

Streptogramins and a process for preparing  
streptogramins by mutasynthesis

The present invention relates principally to novel compounds which are related to the group B streptogramins, and to a process for preparing streptogramins by mutasynthesis. It also relates to novel genes which are involved in the biosynthesis of precursors of the group B streptogramins, and to their uses.

10 The streptogramins form a homogeneous group of antibiotics consisting of an association of two types of chemically different molecules; on the one hand polyunsaturated macrolactones (group A components) and, on the other hand, depsipeptides (group B  
15 components). This group comprises numerous antibiotics which are known under different names according to their origin and includes pristinamycins, mikamycins and virginiamycins (Cocito 1979, 1983).

The A and B components have a synergistic  
20 antibacterial activity which can amount to 100 times that of the separate components and which, contrary to that of each component, is bactericidal (Cocito 1979). This activity is more particularly effective against Gram positive bacteria such as Staphylococci and  
25 Streptococci (Cocito 1979, Videau 1982). Components A and B inhibit protein synthesis by binding to the 50S

subunit of the ribosome (Cocito 1979; Di Giambattista et al., 1989).

While knowledge of the routes by which each of the components is biosynthesized still remains partial to date, earlier studies, presented in Patent Application PCT/FR93/0923, have made it possible to identify several proteins, and the corresponding structural genes, which are involved in the biosynthesis of the two types of component.

Two parts can be distinguished in the process for biosynthesizing group B streptogramins:

1) Biosynthesis of the precursors, or their analogues, of the macrocycle: 3-hydropicolinic acid, L-2-aminobutyric acid, 4-dimethylamino-L-phenylalanine, L-pipecolic acid and L-phenylglycine.

2) Formation of the macrocycle from the precursors listed above, from L-threonine and from L-proline, or their analogues, with (a) possible subsequent modification(s) of the peptide

N-methylation, epimerisation, hydroxylation and oxidation type.

Patent Application PCT/FR93/0923 relates, in particular, to the enzymes which catalyse incorporation of the precursors into the peptide chain of B streptogramins in the process of elongation, and also to their structural genes. These results have demonstrated the non-ribosomal peptide synthesis

character of the type B components.

The present invention relates, more particularly, to novel compounds which are related to group B streptogramins and, more precisely, to novel compounds of the pristinamycin I family (Figures 1 and 2), termed PI below, or of the virginiamycin S family (Figure 3).

The major constituent of the I pristinamycins (PI) is  $PI_A$  (Figure 1), which represents approximately 94% of the PI, with the remaining approximately 6% being represented by minor constituents of the depsipeptide ( $PI_B$  to  $PI_I$ ) whose structures are depicted in Figure 2. PI results essentially from the condensation of amino acids, certain of which are essential for protein synthesis (threonine and proline) and others of which are novel and themselves considered to be secondary metabolites (L-2-aminobutyric acid, 4-dimethylamino-L-phenylalanine (DMPAPA), L-pipecolic acid and L-phenylglycine for  $PI_A$ ), and also of an aromatic precursor, 3-hydroxypicolinic acid.

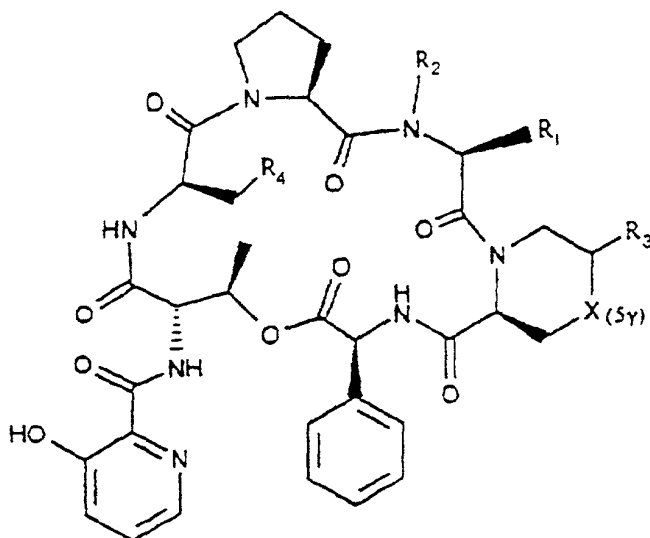
The virginiamycin S derivatives result from condensation of the same acids as in the case of PI, apart from 4-DMPAPA, which is replaced by a phenylalanine (see Figure 3).

Production of these different compounds by biosynthesis therefore requires preliminary synthesis, by a producer strain, of the novel precursors identified above.

The present invention results specifically from a novel process for preparing streptogramins which employs, as a strain for producing streptogramins, a microorganism strain which is mutated so as to alter the biosynthesis of the precursors of the group B streptogramins. According to this process, the said mutant strain is cultured in a medium which is supplemented with a novel precursor which is different from the precursor whose biosynthesis is altered.

Unexpectedly, this results in the production of novel compounds which are related to the group B streptogramins and which are of value in the therapeutic sphere.

More precisely, the present invention relates to novel compounds which are represented by the general formula I:



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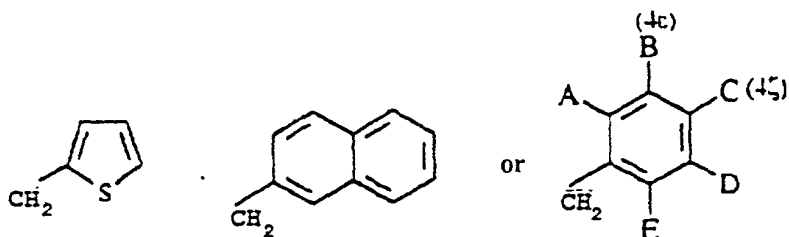
in which:

-  $R_2$  and  $R_4$  represent, independently of each other, a hydrogen atom or a methyl group,

-  $R_3$  represents a hydrogen atom or a hydroxyl group,

5 -  $X$  represents a CO, CHOH or  $CH_2$  group, and

-  $R_1$  represents:



with

- for the meta derivatives:

A, C, D and E representing a hydrogen atom, and

10 B being able to represent

- a halogen, and preferably a fluorine atom,

✓ - a monoalkylamino or dialkylamino group,

with alkyl preferably representing a methyl or ethyl group,

15 - an ether group; more particularly an OR group with R being preferably selected from among the methyl, ethyl, trifluoromethyl and allyl groups,

- a thioether group, preferably represented by an alkylthio group with alkyl preferably

20 representing a methyl group,

- a  $C_1$  to  $C_3$  alkyl group, or

- a trihalogenomethyl group, preferably trifluoromethyl

A, B, D and E representing a hydrogen atom, and  
C being able to represent:

5                   - an  $\text{NR}_1\text{R}_2$  group with  $\text{R}_1$  and  $\text{R}_2$  representing,  
independently of each other, a group selected from  
among

10                                     $\sqrt{-}$  a straight-chain or branched  $C_1$  to  $C_4$   
alkyl group where, when one of the substituents  $R_1$  or  $R_2$   
represents a methyl group, the other necessarily  
represents an ethyl group,

15                                    - an optionally substituted C<sub>3</sub> to C<sub>4</sub>  
cycloalkyl group,

- a substituted or unsubstituted N-pyrrolidinyl group,

- a thioether group, preferably represented by an alkylthio group with alkyl preferably

representing a C<sub>1</sub> to C<sub>3</sub> alkyl group,

- an acyl or alkoxycarbonyl group and, more particularly, a COR group with R preferably representing a C<sub>1</sub> to C<sub>3</sub> alkyl group or a C<sub>1</sub> to C<sub>3</sub> alkoxy group,

- a C<sub>1</sub> to C<sub>6</sub> alkyl group which is straight-chain or branched and which is preferably selected from among the methyl, isopropyl and tert-butyl groups,

- an alkylthiomethyl group and, more preferably, a CH<sub>2</sub>SR group with R preferably representing a C<sub>1</sub> to C<sub>3</sub> alkyl group,

- an aryl group, preferably a phenyl group,

or

- a trihalogenomethyl group, preferably trifluoromethyl

- for the meta-para disubstituted derivatives:

A, D and E representing a hydrogen atom, and

B being able to represent:

- a halogen, preferably a fluorine atom,

- a monoalkylamino or dialkylamino group with alkyl preferably representing a methyl or ethyl group,

- an ether group and preferably an OR group with R preferably selected from among the methyl, ethyl and trifluoromethyl groups,

- a thioether group and preferably alkylthio with alkyl preferably representing an ethyl group, or

- a C<sub>1</sub> to C<sub>3</sub> alkyl group, and

C being able to represent:

- a halogen, preferably a fluorine atom,  
 - an amino, monoalkylamino or dialkylamino  
 group with alkyl preferably representing a methyl group  
 with the proviso that B is different from a bromine or  
 5 chlorine atom, or a substituted or unsubstituted allyl  
 group,

- an ether group and preferably an OR group  
 with R preferably selected from among the methyl, ethyl  
 and trifluoromethyl groups,  
 10 - a thioether group and preferably an  
 alkylthio group with alkyl preferably representing a  
 methyl group,

- a C<sub>1</sub> to C<sub>6</sub> alkyl group, or  
 - a trihalogenomethyl group, preferably  
 15 trifluoromethyl, and  
 - for the ortho-para disubstituted derivatives:  
 B, E and D representing a hydrogen atom and A and C a  
 methyl group.

The following may be more particularly  
 20 mentioned as preferred compounds:

4 $\zeta$ -methylthio-  
 de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub>,  
 4 $\zeta$ -methylthio-  
 de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>B</sub>,  
 25 5 $\gamma$ -hydroxy-4 $\zeta$ -methylthio-de(4 $\zeta$ -  
 dimethylamino)pristinamycin I<sub>B</sub>,  
 4 $\zeta$ -methyl-de(4 $\zeta$ -dimethylamino)pristinamycin  
 I<sub>A</sub>,



- 4 $\zeta$ -methyl-de(4 $\zeta$ -dimethylamino)pristinamycin  
I<sub>H</sub>,
- 4 $\zeta$ -methoxy-de(4 $\zeta$ -dimethylamino)pristinamycin  
I<sub>A</sub>,
- 5 4 $\zeta$ -methoxycarbonyl-de(4 $\zeta$ -  
dimethylamino)pristinamycin I<sub>A</sub>,
- 4 $\zeta$ -chloro-de(4 $\zeta$ -dimethylamino)pristinamycin  
I<sub>A</sub>,
- 10 4 $\zeta$ -bromo-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub>,  
4 $\zeta$ -bromo-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>H</sub>,  
4 $\zeta$ -iodo-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub>,  
4 $\zeta$ -iodo-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>H</sub>,  
4 $\zeta$ -trifluoromethyl-de(4 $\zeta$ -dimethylamino) -  
pristinamycin I<sub>A</sub>,
- 15 4 $\zeta$ -trifluoromethyl-de(4 $\zeta$ -dimethylamino) -  
pristinamycin I<sub>H</sub>,
- 4 $\zeta$ -tert-butyl-de(4 $\zeta$ -dimethylamino) -  
pristinamycin I<sub>A</sub>,
- 4 $\zeta$ -isopropyl-de(4 $\zeta$ -dimethylamino) -  
20 pristinamycin I<sub>A</sub>,
- 4 $\zeta$ -isopropyl-de(4 $\zeta$ -dimethylamino) -  
pristinamycin I<sub>H</sub>,
- 4 $\epsilon$ -methylamino-de(4 $\zeta$ -dimethylamino) -  
pristinamycin I<sub>A</sub>,
- 25 4 $\epsilon$ -methoxy-de(4 $\zeta$ -dimethylamino)pristinamycin  
I<sub>A</sub>,
- 4 $\epsilon$ -methoxy-de(4 $\zeta$ -dimethylamino)pristinamycin  
I<sub>H</sub>,

- 4ε-fluoro 4ζ-methyl-de(4ζ-dimethylamino) -  
 pristinamycin I<sub>A</sub>,  
 4ζ-amino-de(4ζ-dimethylamino)pristinamycin I<sub>A</sub>,  
 4ζ-ethylamino-de(4ζ-dimethylamino) -
- 5 pristinamycin I<sub>A</sub>,  
 4ζ-diethylamino-de(4ζ-dimethylamino) -  
 pristinamycin I<sub>A</sub>,  
 4ζ-allylamino-de(4ζ-dimethylamino) -  
 pristinamycin I<sub>A</sub>,
- 10 4ζ-diallylamino-de(4ζ-dimethylamino) -  
 pristinamycin I<sub>A</sub>,  
 4ζ-allylethylamino-de(4ζ-dimethylamino) -  
 pristinamycin I<sub>A</sub>,  
 4ζ-ethylpropylamino-de(4ζ-dimethylamino) -
- 15 pristinamycin I<sub>A</sub>,  
 4ζ-ethylisopropylamino-de(4ζ-dimethylamino) -  
 pristinamycin I<sub>A</sub>,  
 4ζ-ethylmethylcyclopropylamino-de(4ζ -  
 dimethylamino)pristinamycin I<sub>A</sub>,
- 20 4ζ-(1-pyrrolidinyl) -de(4ζ-dimethylamino) -  
 pristinamycin I<sub>A</sub>,  
 4ζ-trifluoromethoxy-de(4ζ-dimethylamino) -  
 pristinamycin I<sub>A</sub>,  
 4ζ-allyloxy-de(4ζ-dimethylamino)pristinamycin
- 25 I<sub>A</sub>,  
 4ζ-ethoxy-de(4ζ-dimethylamino)pristinamycin  
 I<sub>A</sub>,  
 4ζ-ethylthio-de(4ζ-dimethylamino) -

pristinamycin I<sub>A</sub>,

4ζ-methylthiomethyl-de(4ζ-dimethylamino) -

pristinamycin I<sub>A</sub>,

4ζ-(2-chloroethoxy) -de(4ζ-dimethylamino) -

5 pristinamycin I<sub>A</sub>,

4ζ-acetyl-de(4ζ-dimethylamino)pristinamycin

I<sub>A</sub>,

4ζ-ethyl-de(4ζ-dimethylamino)pristinamycin I<sub>A</sub>,

4ζ-ethyl-de(4ζ-dimethylamino)pristinamycin I<sub>B</sub>,

10 4ε-dimethylamino-de(4ζ-dimethylamino) -

pristinamycin I<sub>A</sub>,

4ε-methylthio-de(4ζ-dimethylamino) -

pristinamycin I<sub>A</sub>,

4ε-ethoxy-de(4ζ-dimethylamino)pristinamycin

15 I<sub>A</sub>.

The present invention is also directed towards a process which is particularly useful for preparing the compounds of the general formula I.

More precisely, it relates to a process for  
20 preparing streptogramins, characterized in that it employs a streptogramin-producing microorganism strain which possesses at least one genetic modification which affects the biosynthesis of a precursor of the group B streptogramins, and in that the said mutant strain is  
25 cultured in a culture medium which is appropriate and which is supplemented with at least one novel precursor which is different from that whose biosynthesis is altered, and in that the said streptogramins are

recovered.

5 The strains which are employed within the scope of the present invention are therefore strains which produce streptogramins and which are mutated. The genetic modification(s) can be located either within one of the genes which is involved in the biosynthesis of the said precursors or outside the coding region, for example in the regions responsible for the expression and/or the transcriptional or post-  
10 transcriptional regulation of the said genes, or in a region belonging to the transcript containing the said genes.

According to one particular embodiment of the invention, the mutant strains possess one or more  
15 genetic modifications within at least one of their genes which is/are involved in the biosynthesis of the group B streptogramin precursors.

This or these genetic modification(s) alter(s) the expression of the said gene, that is  
20 render(s) this gene, and, as the case may be, another of the genes involved in the biosynthesis of the precursors, partially or totally incapable of encoding the natural enzyme which is involved in the biosynthesis of at least one precursor. The inability  
25 of the said genes to encode the natural proteins may be manifested either by the production of a protein which is inactive due to structural or conformational modifications, or by the absence of production, or by

the production of a protein having an altered enzymic activity, or else by the production of the natural protein at an attenuated level or in accordance with a desired mode of regulation. The totality of these possible manifestations is expressed by an alteration of, or perhaps a blockage in, the synthesis of at least one of the group B streptogramin precursors.

The genes which are capable of being mutated within the scope of the present invention are preferably the genes which are involved in the biosynthesis of the following precursors:

L-2-aminobutyric acid, 4-dimethylamino-L-phenylalanine (DMPAPA), L-pipecolic acid, L-phenylglycine and/or 3-hydroxypicolinic acid (3-HPA).

These genes are more preferably the papA, papM, papB (SEQ ID No. 3), papC (SEQ ID No. 2), hpaA (SEQ ID No. 8), snbF (SEQ ID No. 6) and pipA (SEQ ID No. 5) genes described below.

The papA and papM genes have already been described in Patent Application PCT/FR93/0923. They are present on the cosmid pIBV2. The papA gene appears to correspond to a gene for biosynthesizing 4-amino-L-phenylalanine from chorismate. The 4-amino-L-phenylalanine is then dimethylated by the product of the papM gene, an N-methyltransferase, in order to form 4-dimethylamino-L-phenylalanine, DMPAPA, which is then incorporated into pristinamycin I<sub>A</sub>. These two genes are more particularly involved, therefore, in the synthesis

of the precursor termed DMPAPA.

The other genes, papB, papC, pipA, snbF and hpaA, have been identified and characterized within the scope of the present invention. They are grouped together with the snbA, papA and papM genes on a chromosomal region of approximately 10 kb (Figure 7).

The sequence homologies demonstrated for the PapB and PapC proteins show that these proteins are also involved, jointly with the papA and papM proteins, in the biosynthesis of the DMPAPA precursor. The two corresponding novel genes, papB and papC, were isolated and identified by subcloning which was carried out using cosmid pIBV2, described in Patent Application PCT/FR93/0923, and a plasmid, pVRC900, which is derived from pIBV2 by means of a HindIII deletion and is also described in Patent Application PCT/FR93/0923.

The comparison of the protein encoded by the papC gene with the protein sequences contained in the Genpro library shows a 27% homology with the region which is involved in the prephenate dehydrogenase activity of the bifunctional TyrA proteins of E. coli (Hudson and Davidson, 1984) and Erwinia herbicola (EMBL data library, 1991). This region of TyrA catalyses aromatization of the prephenate to form 4-hydroxyphenylpyruvate in the biosynthesis of tyrosine. A similar aromatization, which proceeds from 4-deoxy-4-aminoprephenate and leads to 4-aminophenylpyruvate is very probably involved in the synthesis of

DMPAPA. It would be catalysed by the PapC protein (SEQ ID No. 2).

PapB possesses a 24 to 30% homology with the region which is involved in the chorismate mutase activity of the TyrA and PheA bifunctional proteins of *E. coli* (Hudson and Davidson, 1984) and of the TyrA protein of *Erwinia herbicola*. This region catalyses isomerization of the chorismate to form prephenate in the biosynthesis of tyrosine and of phenylalanine. The PapB protein (SEQ ID No. 3) is probably involved in a similar isomerization which proceeds from 4-deoxy-4-aminochorismate and leads to 4-deoxy-4-aminoprephenate in the synthesis of DMPAPA.

The papA, snbF and hpaA genes have been located in the regions which are contained between the snbA gene, which encodes 3-hydroxypicolinic acid AMP ligase and is described in Patent Application PCT/FR93/0923, and the papA or snbR genes. They were located accurately by means of subcloning, which was carried out using the plasmid pVRC900 and the cosmid pIBV2, which are described in Patent Application PCT/FR93/0923.

On comparing the protein encoded by the hpaA gene and the protein sequences contained in the Genpro library, a homology of from 30 to 40% was detected with a group of proteins which are probably involved (Thorson et al., 1993) in the transamination of intermediates in the biosynthesis of various

antibiotics (DnrJ, EryCl, TylB, StrS and PrgL).

Synthesis of the 3-HPA precursor, which appears to derive from lysine by another route than that of cyclodeamination (see examples 1-2 and 2-1), probably requires a transamination step which can be catalysed by the product of this gene termed hpaA (SEQ ID No. 8). Furthermore, the results of mutating this gene demonstrate unequivocally that it is involved in the synthesis of the 3-HPA precursor.

10                   Comparison of the product encoded by the gene termed pipA with the protein sequences contained in the Genpro library shows a 30% homology with the ornithine cyclodeaminase of Agrobacterium tumefaciens (Schindler et al., 1989). This enzyme is involved in the final  
15                   step of the catabolism of octopine; it converts L-ornithine into L-proline by means of cyclodeamination. Authors have demonstrated, by means of incorporating labelled lysine, that 4-oxopipicolinic acid and 3-hydroxypicolinic acid, which are found both  
20                   in PI<sub>1</sub> and in virginiamycin S1, derived from lysine (Molinero et al., 1989, Reed et al., 1989).  
Cyclodeamination of lysine, in a similar manner to that described for ornithine, would lead to the formation of  
25                   this product was termed PipA (SEQ ID No. 5). The results of mutating the pipA gene, presented in the examples below, demonstrate that it is involved solely in the synthesis of pipicolinic acid. It is noted, in



particular, that this mutation has no effect on the biosynthesis of 3-hydroxypicolinic acid, which is also derived from lysine and of which pipecolic acid could have been a precursor.

5                   Finally, on comparing the product of the gene termed snbF with the protein sequences contained in the Genpro library, a 30 to 40% homology was noted with several hydroxylases of the cytochrome P450 type, which are involved in the biosynthesis of secondary  
10                   metabolites (Omer et al., 1990. Trower et al., 1992). Several hydroxylations can be envisaged in the biosynthesis of the precursors of pristinamycin I, in particular in the biosynthesis of 3-HPA (hydroxylation of picolinic acid at the 3 position) and of  
15                   4-oxopipecolic acid (hydroxylation of pipecolic acid at the 4 position). The corresponding protein was termed SnbF (SEQ ID No. 6).

                  The results of mutating the pipA gene, with polar effects on the expression of the snbF gene,  
20                   demonstrate the involvement of the snbF gene in the hydroxylation of the pipecolic acid residue of group B streptogramins. The expression of the snbF gene is thus altered by the expedient of effecting a genetic modification of the pipA gene.

25                   Preferentially, the genetic modification(s) render(s) the said gene partially or totally incapable of encoding the natural protein.

Genetic modification should be understood to

mean, more particularly, any suppression, substitution, deletion, or addition of one or more bases in the gene(s) under consideration. Such modifications may be obtained in vitro (on the isolated DNA) or in situ, for example, by means of genetic engineering techniques, or else by exposing the said microorganisms to a treatment using mutagenic agents. Examples of mutagenic agents which may be cited are physical agents such as high-energy rays (X,  $\gamma$ , ultra violet, etc. rays), or chemical agents which are able to react with different functional groups of the DNA bases, and, for example, alkylating agents [ethyl methanesulphonate (EMS), N-methyl-N'-nitro-N-nitrosoguanidine, and N-nitroquinoline-1-oxide (NQO)], bialkylating agents, intercalating agents, etc. Deletion is understood to mean any suppression of a part for all of the gene under consideration. This deletion can, in particular, be of a part of the region encoding the said proteins, and/or of all or part of the promoter region for transcription or translation, or else of the transcript.

The genetic modifications may also be obtained by means of gene disruption, for example using the protocol initially described by Rothstein [Meth. Enzymol. 101 (1983) 202] or, advantageously, by means of double homologous recombination. In this case, the integrity of the coding sequence will preferentially be disrupted in order to permit, if need be, replacement,

by means of homologous recombination, of the wild-type genomic sequence with a non-functional or mutant sequence.

According to another option of the invention,  
5 the genetic modifications can consist of placing the gene(s) encoding the said proteins under the control of a regulated promoter.

The mutant microorganism strains according to the present invention may be obtained from any  
10 microorganism which produces streptogramins (cf. Table V). According to one particular embodiment of the invention, the mutant strain is a strain which is derived from *S. pristinaespiralis* and, more particularly, from *S. pristinaespiralis* SP92.

15 Mutant strains which are preferred within the scope of the present invention and which may more particularly be mentioned are the strain SP92::pVRC508, which is mutated in the biosynthesis of the DMPAPA precursor by disrupting the papA gene by means of  
20 simple crossing over, or else, more preferably, the strain SP212, which is mutated in the biosynthesis of the DMPAPA precursor by disrupting the papA gene by means of double homologous recombination. These strains no longer produce PI unless they are supplemented with the  
25 DMPAPA precursor. Unexpectedly, when a novel precursor, which is different from DMPAPA and which is capable, after, in this case, metabolization, of being incorporated by PI synthetase III (SnbD protein which

is responsible for incorporating L-proline and DMPAPA residues) is added to the production medium, these two strains then become able to produce novel I  
 5 produce a component which is normally a minor component of PI, in particular PI<sub>3</sub> (Figure 2).

Two other mutant strains have been prepared within the scope of the present invention. These are, respectively, the strain SP92R, in which the  
 10 pipA gene is disrupted by homologous recombination, and the strain SP92R, in which the hpaA gene is disrupted. While strain SP92R no longer produces PI under standard fermentation conditions, it strongly produces, in the presence of L-pipecolic acid,  
 15 a component, which was initially a minor component among the B streptogramin components, in which 4-oxopipecolic acid is replaced by L-pipecolic acid. While strain *S. pristinaespiralis* SP92R no longer produces PI under standard fermentation  
 20 conditions, it is able to produce novel group B streptogramins in the presence of novel precursors.

By supplementing the medium for culturing mutant strains according to the invention with at least one novel precursor, it turns out that it is possible  
 25 to orient biosynthesis either towards novel streptogramins, or towards a minor form of the streptogramins, or else to favour formation of one of the streptogramins.

The precursors which are employed within the scope of the present invention can be derivatives or analogues of amino acids and, more particularly of phenylalanine, as well as organic acids and, in particular, alpha-cetocarboxylic acids and, more particularly, derivatives of phenylpyruvic acid.

Naturally, the novel precursor is such that it caters for the alteration or blockage, which is induced in accordance with the invention, within the biosynthesis of one of the natural precursors of the group B streptogramins and leads to the synthesis of streptogramins. According to one particular embodiment of the invention, this novel precursor is selected such that it is related to the precursor whose biosynthesis is altered. Thus, in the specific case of the mutant which is blocked in the biosynthesis of DMPAPA, the novel precursor is preferably a derivative of phenylalanine.

The following may, in particular, be cited as precursors which are suitable for the invention:

Phenylalanine, 4-dimethylaminophenylalanine, 4-methylaminophenylalanine, 4-aminophenylalanine, 4-diethylaminophenylalanine, 4-ethylaminophenylalanine, 4-methylthiophenylalanine, 4-methylphenylalanine, 4-methoxyphenylalanine, 4-trifluoromethoxyphenylalanine, 4-methoxycarbonylphenylalanine, 4-chlorophenylalanine, 4-bromophenylalanine, 4-iodophenylalanine,

- 4-trifluoromethylphenylalanine, 4-tert-butylphenylalanine, 4-isopropylphenylalanine, 3-methylaminophenylalanine, 3-methoxyphenylalanine, 3-methylthiophenylalanine, 3-fluoro-
- 5 4-methylphenylalanine, L-pipecolic acid, 4-tert-butylphenylpyruvic acid, 4-methylaminophenylpyruvic acid, 2-naphthylphenylalanine, 4-fluorophenylalanine, 3-trifluorophenylalanine, 3-ethoxyphenylalanine, 2,4-dimethylphenylalanine, 3,4-dimethylphenylalanine,
- 10 3-methylphenylalanine, 4-phenylphenylalanine, 4-butylphenylalanine, 2-thienyl-3-alanine, 3-trifluoromethylphenylalanine, 3-hydroxyphenylalanine, 3-ethylaminophenylalanine, 4-allylaminophenylalanine, 4-diallylaminophenylalanine,
- 15 4-allylethylaminophenylalanine, 4-ethylpropylaminophenylalanine, 4-ethylisopropylaminophenylalanine, 4-ethylmethylcyclopropylaminophenylalanine, 4-(1-pyrrolidinyl)phenylalanine, 4-O-allyltyrosine,
- 20 4-O-ethyltyrosine, 4-ethylthiophenylalanine, 4-ethylthiomethylphenylalanine, 4-O-(2-chloroethyl)tyrosine, 4-acetylphenylalanine, 4-ethylphenylalanine, 3-dimethylaminophenylalanine, 3-ethoxyphenylalanine, 3-fluoro-4-methylphenylalanine
- 25 and 4-aminomethylphenylalanine.

Among these precursors,

4-trifluoromethoxyphenylalanine,  
3-methylaminophenylalanine, 3-methylthiophenylalanine,

3-fluoro-4-methylphenylalanine,  
 4-methylaminophenylpyruvic acid, 3-ethoxyphenylalanine,  
 4-allylaminophenylalanine, 4-diallylaminophenylalanine,  
 4-allylethylaminophenylalanine,  
 5 4-ethylpropylaminophenylalanine,  
 4-ethylisopropylaminophenylalanine,  
 4-ethylmethylcyclopropylaminophenylalanine,  
 4-(1-pyrrolidiny1)phenylalanine,  
 4-ethylthiomethylphenylalanine,  
 10 4-O-(2-chloroethyl)tyrosine,  
 3-dimethylaminophenylalanine and  
 3-ethylaminophenylalanine are novel and were prepared  
 and characterized within the scope of the present  
 invention. They are found to be particularly useful for  
 15 preparing streptogramins according to the invention.

The claimed process turns out to be  
 particularly advantageous for preparing novel group B  
 streptogramins or else for favouring formation of  
 particular streptogramins. As such, it is particularly  
 20 useful for preparing PIB.

The present invention also relates to a  
 nucleotide sequence which is selected from among:

(a) all or part of the genes papC (SEQ ID  
 No. 2), papB (SEQ ID No. 3), pipA (SEQ ID No. 5), snbF  
 25 (SEQ ID No. 6) and hpaA (SEQ ID No. 8),

(b) sequences which hybridize with all or  
 part of the (a) genes, and

(c) sequences which are derived from (a) and

(b) sequences on account of the degeneracy of the genetic code.

In the particular case of the hybrid sequences according to (b), these sequences preferably  
5 encode a polypeptide which is involved in the biosynthesis of the streptogramins.

Still more preferably, the invention relates to the nucleotide sequences which are represented by the genes papC (SEQ ID No. 2), papB (SEQ ID No. 3),  
10 pipA (SEQ ID No. 5), snbF (SEQ ID No. 6), and hpaA (SEQ ID No. 8).

The invention furthermore relates to any recombinant DNA which encompasses a papC (SEQ ID No. 2), papB (SEQ ID No. 3), pipA (SEQ ID No. 5), snbF (SEQ  
15 ID No. 6) or hpaA (SEQ ID No. 8) gene.

Naturally, the nucleotide sequences defined above can be part of a vector of the expression vector type, which can be an autonomously replicating vector, an integrated vector or a suicide vector. The present  
20 invention is also directed to these vectors as well as to any use of a sequence according to the invention or of a corresponding vector for, in particular, preparing metabolites of interest. It furthermore relates to any polypeptide which results from the expression of a  
25 claimed sequence.

The present invention also relates to any mutated S. pristinaespiralis strain which possesses at least one genetic modification within one of the papC



(SEQ ID No. 2), papB (SEQ ID No. 3), pipA (SEQ ID No. 5), snbF (SEQ ID No. 6) and hpaA (SEQ ID No. 8) genes, and, more preferably, to strains SP92pipA:: $\Omega$ am<sup>R</sup> and SP92hpaA:: $\Omega$ am<sup>R</sup>, as well as any *S. pristinaespiralis* strain, such as SP212, which possesses a genetic modification which consists of a disruption of the papA gene by means of double homologous recombination.

Combinations of a component of the group A streptogramins and of a compound of the general formula I, according to the invention, constitute compositions which are particularly advantageous in the therapeutic sphere. They are employed, in particular, for treating ailments which are due to Gram-positive bacteria (of the genre *Staphylococci*, *Streptococci*, *Pneumococci* and *Enterococci*) and Gram-negative bacteria (of the genre *Haemophilus*, *Gonococci*, *Meningococci*). Thus, the compounds according to the invention have a synergistic effect on the antibacterial action of pristinamycin IIB on *Staphylococcus aureus* IP8203 in mice in vivo, at oral doses which are principally between 30 mg/kg and 100 mg/kg, when they are combined in PI/PII proportions of the order of 30/70.

The present invention extends to any pharmaceutical composition which contains at least one compound of the general formula I which is or is not combined with a group A streptogramin.

The examples appearing below are presented by way of illustrating the present invention and do not

limit it.

LIST OF FIGURES.

- Figure 1: Structure of pristinamycin I<sub>A</sub>.
- Figure 2: Structure of the minor components of pristinamycin I.
- Figure 3: Other examples of structures of B components of streptogramins.
- Figure 4: Depiction of the PstI-XhoI region of 2.9 kb.
- Figure 5: Depiction of the XhoI-PstI region of 4.5 kb.
- Figure 6: Depiction of the HindIII-BglII region of 1.6 kb.
- Figure 7: Depiction of the BglII-XhoI region of approximately 10 kb.
- Figure 8: Depiction of plasmid pVRC415.
- Figure 9: Depiction of plasmid pVRC420.
- Figure 10: Depiction of plasmid pVRC411.
- Figure 11: Depiction of plasmid pVRC421.
- Figure 12: Depiction of plasmid pVRC414.
- Figure 13: Strategy for constructing SP212.

**EXAMPLE 1: Sequencing and identification of genes involved in the biosynthesis of pristinamycin I and its precursors.**

Identification, by means of sequencing, of the genes situated downstream and upstream of the gene which encodes the enzyme PapA and which is described in Patent PCT/FR93/0923, as well as of a gene which is

situated downstream of the gene which encodes the enzyme *SnbA* and which is also described in Patent PCT/FR93/0923.

This example describes how, using cosmid  
5 pIBV2, which is described in Patent PCT/FR93/0923 and which contains the structural genes for the enzymes *PapA* and *PapM*, which are involved in the synthesis of the 4-dimethylamino-L-phenylalanine (DMPAPA) precursor of pristinamycin I, and the structural gene for the  
10 enzyme *SnbA*, which is responsible for activating the aromatic precursor, 3-hydroxypicolinic acid (3-HPA), of pristinamycin I, it proved possible to identify, by sequencing around these genes and studying the corresponding mutants, other genes which are involved  
15 in the biosynthesis of the DMPAPA precursor or in the biosynthesis of other precursors of pristinamycin I.

With this aim in mind, subclonings were carried out using cosmid pIBV2 and plasmid pVRC900, which is derived from pIBV2 by means of a HindIII  
20 deletion and which is also described in Patent PCT/FR93/0923.

This example illustrates how the nucleotide sequences of fragments situated downstream and upstream of the papA and snbA genes of *S. pristinaespiralis* can  
25 be obtained.

The techniques for cloning DNA fragments of interest in the M13mp18 and 19 vectors (Messing et al. 1981) are standard techniques for cloning in

Escherichia coli and are described in Maniatis et al. (1989).

1-1 Sequencing and analysis of the region downstream of the papA gene

5 In order to sequence this region, which is contained between the papA and papM genes, the PstI-PstI fragment of 1.5 kb, the PstI-XhoI fragment of 0.7 kb, and the XhoI-XhoI fragment of 0.7 kb were subcloned into the M13mp18 and M13mp19 vectors  
10 proceeding from plasmid pVRC900. The cloning sites were sequenced through by sequencing on double-stranded DNA using plasmids pVRC900 and pVRC409, which are described in Patent PCT/FR93/0923.

The clonings were carried out as follows.

15 Approximately 2  $\mu$ g of plasmid pVRC900 were cut with restriction enzymes PstI and/or XhoI (New England Biolands) under the conditions recommended by the supplier. The restriction fragments thus obtained were separated on a 0.8% agarose gel, and the 1.5 kb  
20 PstI-PstI, 0.7 kb PstI-XhoI and 0.7 kb XhoI-XhoI fragments of interest were isolated and purified using GeneClean (Bio101, La Jolla, California). For each cloning, approximately 10 ng of M13mp19 and/or M13mp18, cut with PstI and/or XhoI, were ligated to 100 ng of  
25 the fragment to be cloned under the conditions described by Maniatis et al. 1989. After transforming the strain TG1 (K12,  $\Delta$ (lac-pro) *supE thi hsd*  $\Delta$ S F' *traD36 proA<sup>+</sup>B<sup>+</sup> lacI<sup>s</sup> lacZ*  $\Delta$  M15; Gibson, 1984) and

selecting lysis plaques on an LB + X-gal + IPTG medium in accordance with the technique described by Maniatis et al. (1989), the phage carrying the desired fragments were isolated. The different inserts were sequenced by the chain termination reaction using, as the synthesis primer, the universal primer or synthetic oligonucleotides which were complementary to a 20 nucleotide sequence of the insert to be sequenced. The reactions were carried out using fluorescent dideoxynucleotides (PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit-Applied Biosystem) and analysed on a model 373 A Applied Biosystems DNA sequencer. The overlap between these different inserts was such that it was possible to establish the entire nucleotide sequence between the papA and papM genes (SEQ ID No. 1).

With the aid of this nucleotide sequence, it is possible to determine the open reading frames and thereby identify genes which are involved, in S. pristinaespiralis, in the biosynthesis of PI or its precursors, as well as the polypeptides encoded by these genes.

We looked for the presence of open reading frames within the 2.9 kb PstI-XhoI fragment, which contains the nucleotide sequence between the papA and papM genes, making use of the fact that *Streptomyces* DNA displays a high percentage of G and C bases as well as a strong bias in the use of codons which make up the

coding frames (Bibb et al. 1984). The method of Staden and McLachlan (1982) makes it possible to calculate the probability of coding frames in terms of the codon usage of Streptomyces genes which have already been  
5 sequenced and which are assembled in a data file which contains 19673 codons and which was obtained using the BISANCE (Dessen et al. 1990) computer server.

Using this method, it was possible to characterize four highly probable open reading frames  
10 within the 2.9 kb PstI-XhoI fragment, which reading frames are depicted in the table below (TABLE I). They are designated frames 1 to 4 according to their position starting from the PstI site. The length of each reading frame in bases, has been indicated, as has  
15 its position within the fragment (the PstI site being situated at position 1); the number of amino acids in the encoded polypeptide has also been indicated for open reading frames 2 and 3. Frames 1, 3 and 4 are encoded by the same strand, while frame 2 is encoded by  
20 the complementary strand (Figure 4). Frames 1 and 4 correspond, respectively, to the C-terminal region of the PapA protein and to the N-terminal region of the PapM protein, which proteins were previously identified and described in Patent PCT/FR93/00923.

Frame number and/or gene name	Position	Number of nucleotides	Number of amino acids
1 (PapA)	1-684	684	-
5 2 (PapC) (inv)	949-1836	888	296
3 (PapB)	1873-2259	387	129
4 (PapM)	2259-2887	629	-

TABLE I

Comparison of the product of frame 2 (TABLE I) with the protein sequences contained in the Genpro library shows a 27% homology with the region involved in the prephenate dehydrogenase activity of the bifunctional TyrA proteins of E. coli (Hudson and Davidson, 1984) and of Erwinia herbicola (EMBL data library, 1991). This region of TyrA catalyses aromatization of prephenate to form 4-hydroxyphenylpyruvate in the biosynthesis of tyrosine. A similar aromatization, proceeding from 4-deoxy-4-aminoprephenate and leading to 4-aminophenylpyruvate is very probably involved in the synthesis of DMPAPA. This reaction will be catalysed by the product of frame 2, termed PapC (SEQ ID N . 2).

Comparison of the product of frame 3 (TABLE I) with the protein sequences contained in the

Genpro library shows a 24 to 30% homology with the region involved in the chorismate mutase activity of the bifunctional TyrA and PheA proteins of E. coli (Hudson and Davidson, 1984) and of the TyrA protein of Erwinia herbicola. This region catalyses isomerization of chorismate to form prephenate in the biosynthesis of tyrosine and phenylalanine. A similar isomerization, proceeding from 4-deoxy-4-amino chorismate and leading to 4-deoxy-4-aminoprephenate, is very probably involved in the synthesis of DMPAPA. This reaction would be catalysed by the product of frame 3, termed PapB (SEQ ID No. 3).

In the case of TyrA and PheA, the chorismate mutase and prephenate dehydratase, or prephenate dehydrogenase, activities are catalysed by the same protein. In S. pristinaespiralis, the chorismate mutase and prephenate dehydrogenase enzyme activities are catalysed by two separate proteins, i.e. PapB and PapC, respectively.

The sequence homologies demonstrated for the PapB and PapC proteins demonstrate that these two proteins are involved, jointly with the PapA and PapM proteins, in the biosynthesis of the aromatic derivative DMPAPA. In the same way as for papA, disruption of the papB and papC genes should lead to the construction of S. pristinaespiralis strains which are incapable of producing PI but which are able, in the presence of novel precursors, to produce new PIs



which are modified at the level of the DMPAPA residue.

1-2. Sequencing and analysis of the region upstream of the papA gene

This region is contained between the snbA gene, which encodes 3-hydroxypicolinic acid AMP ligase and which is described in Patent PCT/FR93/00923, and the papA gene.

The clonings were carried out as described in Example 1-1, proceeding from plasmid pVRC900 and cosmid pIBV2, which are described in Patent PCT/FR93/00923. The 1.3 kb XhoI-XhoI, 0.2 kb XhoI-XhoI, 3.3 kb XhoI-XhoI, 1.1 kb HindIII-PstI and 2.2 kb PstI-PstI fragments were subcloned into the M13mp18 and M13mp19 vectors. These different clonings made it possible to pass through all the cloning sites. The different inserts were sequenced as described in 1-1 using, as synthesis primer, the universal primer or synthetic oligonucleotides which were complementary to a 20 nucleotide sequence in the insert to be sequenced.

The overlap between these different inserts enabled the entire nucleotide sequence which is present between the snbA and papA genes (SEQ ID No. 4) to be established.

On the basis of this nucleotide sequence, it is possible to determine the open reading frames and to identify genes which are involved, in S. pristinaespiralis, in the biosynthesis of precursors of PI, as well as the polypeptides encoded by these

genes.

We have looked for the presence of open reading frames within the 4.5 kb XhoI-PstI fragment, which contains the nucleotide sequence between the snbA and papA genes, as described in Example 1.1. Using this method, it was possible to characterize four highly probable open reading frames within the 4.5 kb XhoI-PstI fragment, which frames are depicted in the table below (TABLE II). They are designated frames 1 to 4 in accordance with their position starting from the XhoI site. Their length in bases, and their position within the fragment (the XhoI site being situated at position I) has been indicated for each fragment; the number of amino acids within the encoded polypeptide has also been indicated for open reading frames 2 and 3. Frames 2, 3 and 4 are encoded by the same strand, and frame 1 is encoded by the complementary strand (Figure 5). Frames 1 and 4 correspond, respectively, to the N-terminal regions of the SnbA and PapA proteins, which were previously identified and described in patent PCT/FR93/00923.

Frame number and/or gene name	Position	Number of nucleotides	Number of amino acids
1 (SnbA) (inv)	1-329	329	-
5 2 (PipA)	607-1671	1065	355
3 (SnbF)	1800-2993	1194	398
4 (PapA)	3018-4496	1479	-

TABLE II

## Comparison of the product of frame 2

10 (TABLE II) with the protein sequences contained in the Genpro library shows a 30% homology with ornithine cyclodeaminase of Agrobacterium tumefaciens (Schindler et al., 1989). This enzyme is involved in the final step in the catabolism of octopine; it converts

15 L-ornithine into L-proline by means of cyclodeamination. Authors have demonstrated, by means of the incorporation of labelled lysine, that 4-oxopipicolinic acid and 3-hydroxypicolinic acid, which are found both in PI<sub>1</sub> and in virginiamycin S1, derived

20 from lysine (Molinero et al., 1989; Reed et al., 1989). A reaction in which lysine was cyclodeaminated, similar to that described for ornithine, would lead to the formation of pipicolinic acid. Taking this hypothesis into account, the product of frame 2 was termed PipA

(SEQ ID No. 5). The results of mutating the pipA gene, presented in 2-1, demonstrate that the pipA gene is involved solely in the synthesis of pipecolic acid, since this mutation has no effect on the biosynthesis of 3-hydroxypicolinic acid, which is also derived from lysine and of which pipecolic acid could have been a precursor.

Comparison of the product of frame 3 (TABLE II) with the protein sequences contained in the Genpro library shows a 30 to 40% homology with several hydroxylases of the cytochrome P450 type, which hydroxylases are involved in the biosynthesis of secondary metabolites (Omer et al., 1990, Trower et al., 1992). Several hydroxylations can be envisaged in the biosynthesis of precursors of pristinamycin I, in particular in the biosynthesis of 3-HPA (hydroxylation of picolinic acid at the 3 position) and of 4-oxopipecolic acid (hydroxylation of pipecolic acid at the 4 position). The results of mutating the pipA gene, presented in 2-1-3, demonstrate that the product of frame 3 is involved in hydroxylation of the pipecolic acid residue of PI<sub>2</sub>. The corresponding gene has therefore been termed snbF, and the corresponding protein SnbF (SEQ ID No. 6).

1-3. Sequencing the region downstream of the snbA gene.

This region is included between the snbA gene, which encodes 3-hydroxypicolinic acid adenylate

ligase, and the snbR gene, which encodes a membrane protein which is probably responsible for transport and for resistance to PI, with both genes having been described in Patent PCT/FR93/00923. Sequencing of this region was carried out using a fragment which was isolated from cosmid pIBV2, as described in Example 1-1.

The 1.6 kb HindIII-BglII fragment was subcloned into the M13mp18 and M13mp19 vectors, proceeding from cosmid pIBV2. The insert was sequenced as described in 1-1, using, as synthesis primer, the universal primer or synthetic oligonucleotides which were complementary to a 20 nucleotide sequence of the insert to be sequenced. On the basis of the nucleotide sequence thus obtained (SEQ ID No. 7), it is possible to determine the open reading frames and to identify, in S. pristinaespiralis, genes which are involved in the biosynthesis of the precursors of PI, as well as the polypeptides encoded by these genes. We looked for the presence of open reading frames within the 1.6 kb HindIII-BglII fragment, which corresponds to the end of the snbA gene and its downstream region, as described in Example 1-1. A complete open coding frame, encoded by the same strand as the snbA gene (Figure 6), was detected. Relative to position 1, corresponding to the HindIII site, this frame starts at nucleotide 249, i.e. 30 nucleotides after the end of the snbA gene, and terminates at nucleotide 1481. It is 1233 nucleotides

in size, corresponding to a protein of 411 amino acids.

Comparison of the product of this open frame with the protein sequences contained in the Genpro library shows a 30 to 40% homology with a group of proteins which are probably involved (Thorson *et al.*, 1993) in the transamination of intermediates in the biosynthesis of various antibiotics (DnrJ, EryC1, TylB, StrS and PrgL). Synthesis of the 3-HPA precursor, which appears to derive from lysine by a route other than cyclodeamination (see Examples 1-2 and 2-1), could necessitate a transamination step which can be catalysed by the product of this frame 3, termed HpaA (SEQ ID No. 8). The results of mutating this gene, presented in 2-2, demonstrate unequivocally that this gene is involved in synthesis of the 3-HPA precursor and confirm our hypothesis.

The genes papB, papC, pipA, snbF and hpaA, which are described in the present invention, are grouped together with the snbA, papA and papM genes on a chromosomal region of approximately 10 kb (Figure 7). This confirms the presence of a cluster of genes which are involved in the biosynthesis of PI and its precursors. Studying regions upstream and downstream of this cluster should enable the other genes involved in the biosynthesis of PI precursors, in particular L-phenylglycine and L-2-aminobutyric acid, to be identified.

**EXAMPLE 2: Construction of recombinant strains by means of disrupting identified genes.**

This example illustrates how it is possible to demonstrate involvement of the genes described in Example 1 in the biosynthesis of pristinamycin precursors, and also to construct S. pristinaespiralis strains which are able to produce novel pristinamycins. These strains are obtained by disrupting the genes which are involved in the biosynthesis of the residue which it is desired to replace, and the novel pristinamycins are produced by supplementing these mutants with novel precursors.

Strain SP92::pVRCC508, which is employed in the present invention to produce novel derivatives of PI by replacing the precursor DMPAPA with other molecules, is described in Patent PCT/FR93/0923. It is obtained by disrupting, by means of simple crossing over, the papA gene, which is involved in the biosynthesis of the precursor of DMPAPA and is thought to participate in an early step relating to the transamination of chorismate. This disruption has a polar character since, in this mutant, expression of the papM gene (PCT/FR93/0923), which is situated 1.5 kb downstream of the papA gene and is involved in the double methylation of 4-amino-L-phenylalanine to form DMPAPA, is very reduced. Thus, assaying the activity of the SAM-dependant methylation enzyme for converting 4-amino-L-phenylalanine (PAPA) into DMPAPA indicates that

mutant SP92::pVRC508 has an activity which is less than 5% of the activity of the wild-type strain.

In the present invention, this strain, SP92::pVRC508, can be used, under appropriate  
 5 fermentation conditions and supplementation conditions, to produce novel pristinamycins which are modified at the level of the DMPAPA residue, as will be explained in Example 3. Mutants having the same phenotype can be obtained by disrupting the papB or papC genes described  
 10 in the present invention.

Another type of S. pristinaespiralis strain, whose papA gene is disrupted and which possesses the same phenotype as strain SP92::pVRC508, was obtained in a similar manner by disrupting the papA gene by means  
 15 of double crossing over. This construction was carried out starting with a 4.6 kb SphI-HindIII fragment, which fragment was isolated from cosmid pIBV2 and contains the 3' region of the pipA gene, the entire snbF and papA genes and the 3' part of the papC gene. This  
 20 fragment was cloned into the suicide vector pDH5, which vector is only able to replicate in E. coli but carries a resistance marker which is expressed in Streptomyces (the gene for resistance to thiostrepton or to nohiheptide, tsr). This vector, pDH5, was developed by  
 25 Wohleben et al (1991 Nucleic Acid Res. 19, 727-731). A BclI-BclI deletion of 1.1 kb was then made in the papA gene, and a 2.2 kb HindIII-HindIII fragment, carrying the amR gene (resistance to geneticin and to



apramycin), was introduced after the cohesive ends had been filled in. The recombinant vector was termed pVRC414 and is depicted in Figure 12. After transforming the pristinamycin-producing strain with  
 5 plasmid pVRC414, transformants which were resistant to geneticin and sensitive to thiostrepton were isolated and analysed. These clones are the result of a double homologous recombination between the  
 10 *S. pristinaespiralis* DNA regions of plasmid pVRC414 and the corresponding chromosomal region of  
*S. pristinaespiralis*, as described in Figure 13. One of these clones was termed SP212. Its phenotype is identical to that of strain SP92::pVRC508 as regards the absence of any production of PI and the ability of  
 15 the strain to produce new antibiotics in the presence of novel precursors. Advantageously, this type of strain, which is obtained by double crossing over, is more stable than the strains which are obtained by simple crossing over.

20                   2-1. Construction of a mutant of  
*S. pristinaespiralis* SP92 whose pipA gene is disrupted.

This example illustrates how it is possible, by means of disrupting the pipA gene, to construct a strain of *S. pristinaespiralis* SP92 which no longer  
 25 produces PI under standard fermentation conditions and which is able to produce new pristinamycins, which are modified at the level of the 4-oxopipicolinic acid residue of PIA, when novel precursors are added to the

fermentation.

It was constructed using a suicide vector, the vector pUC1318, which only replicates in E. coli. This vector does not carry any resistance marker which is expressed in *Streptomyces*. Its presence in the genome of *Streptomyces* can only be detected by colony hybridization.

#### 2-1-1. Construction of plasmid pVRC420:

This example illustrates how it is possible to construct a plasmid which does not replicate in S. pristinaespiralis SP92 and which can be employed to disrupt the pipA gene by means of double homologous recombination.

Plasmid pVRC420 was constructed in order to produce the chromosomal mutant of SP92 in which the pipA gene is disrupted, proceeding from cosmid pIBV2, which is described in Patent PCT/FR93/0923. Cosmid pIBV2 was cut with the restriction enzyme PstI and, after the fragments, thus generated, had been separated by electrophoresis on a 0.8% agarose gel, a 2.8 kb PstI-PstI fragment, containing the start of the snbA and snbF genes and the whole of the pipA gene, was isolated and purified using Geneclean (Bio101, La Jolla, California). 50 ng of vector pUC1318, which had been linearized by digesting with PstI, were ligated to 200 ng of the 2.8 kb fragment, as described in Example 1. A clone carrying the desired fragment was isolated following transformation of the strain TG1 and

selection on LB + 150  $\mu$ g/ml ampicillin + X-gal + IPTG medium. The recombinant plasmid was termed pVRC415 (Figure 8). A cassette containing the am<sup>R</sup> gene, encoding resistance to apramycin or to geneticin (Kuhstoss *et al.*, 1991), was then introduced into the unique HindIII site of plasmid pVRC415, this site being situated 530 bp downstream of the start of the pipA gene. This construction was effected as follows. A 2.5 kb DNA fragment, containing the am<sup>R</sup> gene, the Perme promoter (Bibb *et al.*, 1985) and the first 158 amino acids of the gene for resistance to erythromycin, ermE, was isolated by means of a SalI-BglII double digestion of a plasmid which was derived from plasmids pIJ4026 (plasmid carrying the ermE gene under the control of the Perme promoter) and pHP45 $\Omega$ am<sup>R</sup>. After filling in the SalI and BglII protruding 5' cohesive ends using Klenow enzyme in accordance with the protocol described by Maniatis *et al.*, 1989, the fragment containing the am<sup>R</sup> gene was cloned into the HindIII site of plasmid pVRC415, whose protruding 5' cohesive ends had also been filled in with Klenow enzyme as previously described. The recombinant plasmid thus obtained was designated pVRC420. Its restriction map is depicted in Figure 9.

2-1-2. Isolation of mutant SP92pipA:: $\Omega$ am<sup>R</sup>, whose pipA gene is disrupted by homologous recombination.

This example illustrates how the mutant of

S. pristinaespiralis SP92 whose pipA gene is disrupted was constructed.

This mutant was isolated by transforming strain SP92 with the suicide plasmid pVRC420.

5           The preparation of protoplasts, their transformation and extraction of the total DNA from the recombinant strains were all effected as described by Hopwood et al. (1985).

10           The strain SP92 was cultured, at 30°C for 40 hours, in YEME medium (Hopwood et al., 1985), 34% sucrose, 5 mM MgCl<sub>2</sub> and 0.25% glycine. The mycelium was protoplasted in the presence of lysozyme, and 5 × 1 µg of pVRC420 were used to transform (by the method employing PEG) the protoplasts. After one night in  
15           which the protoplasts were regenerated on R2YE medium (D. Hopwood et al. 1985), the recombinants were selected by spreading on 3 ml of SNA medium (D. Hopwood et al. 1985) containing 1,500 µg/ml geneticin.

20           100 clones which were resistant to geneticin were isolated from the 5 transformations that were carried out. These recombinants arise from integration, by means of simple or double homologous recombination between the pipA gene which is carried by the chromosome of strain SP92 and the parts of the pipA  
25           gene which are contained in the 5.3 kb fragment carried by the suicide plasmid pVRC420. In order to select the recombinants which were obtained by double crossing over (that is which did not contain the pUC1318 part of

plasmid pVRC420 in their genome), colony hybridizations were carried out on 90 clones using pUC19 labelled with [ $\alpha$ - $^{32}$ P]dCTP as the probe, as described in Maniatis et al (1989). 10 clones were selected which were resistant to geneticin but which did not hybridize the vector pUC19. The spores of the recombinants were isolated by streaking and growing on HT7 medium containing 10  $\mu$ g/ml geneticin, and restreaked on the same medium in order to obtain isolated colonies. In order to verify the position at which plasmid pVRC420 was integrated, various Southernns of the total DNA from several recombinant clones, purified as described by Hopwood et al. 1985, were carried out, with hybridization to the 2.8 kb PstI-PstI fragment, which was used as a probe after having been labelled with [ $\alpha$ - $^{32}$ P]dCTP. The results confirm that these recombinants were obtained by double crossing over between vector pVRC420 and the chromosome of strain SP92, resulting in replacement of the 2.8 kb PstI-PstI fragment, containing the pipA gene, by a 5.3 kb PstI-PstI fragment containing the pipA gene which is disrupted by introduction of the am<sup>R</sup> gene. One of these mutants was designated SP92pipA::am<sup>R</sup>.

2-1-3. Production of pristinamycins using mutant SP92pipA::am<sup>R</sup>.

This example illustrates how it is established that the mutant of S. pristinaespiralis SP92 whose pipA gene is disrupted by integration of plasmid pVR420 on the one hand no longer produces PI

under standard fermentation conditions and on the other hand exhibits a high level of production of a minor form of the B components of streptogramins in which 4-oxopipecolic acid is replaced by pipecolic acid.

5                    Mutant SP92pipA::Ωam<sup>R</sup>, as well as strain SP92 in the role of a control strain, were cultured in liquid production medium. The fermentation was carried out as follows: 0.5 ml of a suspension of spores from the abovementioned strain are added, under sterile  
10 conditions, to 40 ml of inoculum medium in a 300 ml baffled Erlenmeyer flask. The inoculum medium is made up of 10 g/l corn steep, 15 g/l sucrose, 10 g/l (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1 g/l K<sub>2</sub>HPO<sub>4</sub>, 3 g/l NaCl, 0.2 g/l MgSO<sub>4</sub>-7H<sub>2</sub>O and 1.25 g/l CaCO<sub>3</sub>. The pH is adjusted to 6.9 using  
15 sodium hydroxide solution before introducing the calcium carbonate. The Erlenmeyer flasks are shaken at 27°C for 44 h on a rotating shaker at a speed of 325 rpm. 2.5 ml of the previous culture, which is 44 fold, are added under sterile conditions to 30 ml of  
20 production medium in a 300 ml Erlenmeyer flask. The production medium is made up of 25 g/l soya flour, 7.5 g/l starch, 22.5 g/l glucose, 3.5 g/l fodder yeast, 0.5 g/l zinc sulphate and 6 g/l calcium carbonate. The pH is adjusted to 6.0 with hydrochloric acid before  
25 introducing the calcium carbonate. The Erlenmeyer flasks are shaken for 24, 28 and 32 hours at 27°C. At each time point, 10 g of must are weighed into a smooth Erlenmeyer flask to which 20 ml of mobile phase,

consisting of 34% of acetonitrile and 66% of a solution of 0.1 M  $\text{KH}_2\text{PO}_4$  (adjusted to pH 2.9 with concentrated  $\text{H}_3\text{PO}_4$ ) are added for extracting the pristinamycins.

- After shaking, the whole is centrifuged and the
- 5 pristinamycins contained in the supernatant are assayed by HPLC by means of injecting 150  $\mu\text{l}$  of the centrifugation supernatant onto a Nucleosil 5-C8 column of 4.6  $\times$  150 mm, which is eluted with a mixture of 40% acetonitrile and 60% 0.1 M phosphate buffer, pH 2.9.
- 10 The I pristinamycins are detected by means of their UV absorbance at 206 nm.

- The results demonstrated that, under the fermentation conditions employed, mutant SP92

pipA:: $\Omega$ am<sup>R</sup> did not produce PI at 24, 28 or 32 hrs of fermentation,

15 while control strain SP92 produced a quantity of PI which was standard for the 3 times which were tested. The quantity of PII which was produced remained the same for the two strains. Mutant SP92

pipA:: $\Omega$ am<sup>R</sup> is definitely blocked at a step in the biosynthesis of PI.

20 Fermentation complementation tests were carried out by adding different precursors of PI, separately or together, to the culture in production medium after 16 hours. The results of these complementations demonstrated that when 100 mg/l pipercolic acid and

25 100 mg/l DMPAPA are added simultaneously to the fermentation medium, the mutant produces what is normally a minor derivative of PI, i.e. PI<sub>2</sub> (which is produced by SP92 in a quantity which is less than 5%)

[illegible]



inhibition of the function of hydroxylation of the  
 pipecolic acid residue of  $PI_x$  to form 4-hydroxypipecolic  
 acid, which is found in  $PI_y$  and  $PI_c$  (Figure 2) and then  
 oxidized to 4-oxopipecolic acid in  $PI_A$ .

5               Preparing a mutant of this nature made it  
 possible to construct a strain of S. pristinaespiralis  
 which is unable to produce PI except in the presence of  
 the PI precursors DMPAPA and pipecolic acid, using  
 which it is able to produce, in a quantity equivalent  
 10 to that of the starting strain, what is normally a  
 minor derivative of PI within the pristinamycin  
 mixture. Similarly, in the presence of novel  
 precursors, or of a mixture of novel precursors and of  
 precursors which are normally present in PI, this  
 15 strain will be able to produce new pristinamycins which  
 are modified in either DMPAPA or 4-oxopipecolic acid or  
 in both these residues.

2-2. Construction of a mutant of  
S. pristinaespiralis SP92 whose hpaA gene is disrupted.

20               This example illustrates how it is possible,  
 by means of disrupting the hpaA gene, to construct a  
 strain of S. pristinaespiralis SP92 which no longer  
 produces PI under standard fermentation conditions and  
 which is able to produce new pristinamycins, which are  
 25 modified at the level of the 3-HPA precursor, when  
 novel precursors are added to the fermentation.

This mutant was constructed using a plasmid  
 which does not replicate in S. pristinaespiralis SP92

and which can be used for disrupting the hpaA gene by means of double homologous recombination.

2-2-1. Construction of the suicide plasmid pVRC421

5                   Plasmid pVRC421 was constructed using a suicide vector which, while only being able to replicate in E. coli, carries a resistance marker which is expressed in *Streptomyces*, i.e. the gene for resistance to thiostrepton or to nosiheptide, tsr. This  
10   vector, pDH5, was developed by Hillemann et al. (1991).

Plasmid pVRC421 was constructed in order to produce the chromosomal mutant of SP92 whose hpaA gene is disrupted, making use of cosmid pIBV2, which is described in Patent PCT/FR93/0923. pIBV2 was digested  
15   with the restriction enzyme SphI and, after having separated the fragments, thus generated, by means of electrophoresis on a 0.6% agarose gel, a 4.8 kb SphI-SphI fragment, containing the whole of the hpaA gene and virtually the whole of the snbA gene, was  
20   isolated and purified using Geneclean as described above. 50 ng of the vector pDH5, linearized by digesting with SphI, were ligated to 200 ng of the 4.8 kb fragment, as subsequently described. A clone harbouring the desired fragment was isolated after  
25   transforming the strain TGl and selecting on LB + 150 µg/ml ampicillin + IPTG + X-gal medium. The recombinant plasmid was designated pVRC411 (Figure 10). A cassette containing the gene am<sup>R</sup>, encoding resistance

to apramycin or to geneticin, was then introduced into the unique PflmI site of plasmid pVRC411, this site being situated 610 bp downstream of the start of the hpaA gene. This construct was produced as follows. A

5 2.2 kb DNA fragment, containing the am<sup>R</sup> gene, was isolated following digestion of the plasmid pHP45am<sup>R</sup>, containing the am<sup>R</sup> gene, with HindIII. After filling in the HindIII protruding 5' cohesive ends using Klenow enzyme according to the protocol described by Maniatis

10 et al. 1989, the fragment containing the am<sup>R</sup> gene was cloned into the PflmI site of plasmid pVRC411, whose protruding 3' cohesive ends had been rendered blunt using the enzyme T4 polymerase as described in Maniatis et al. 1989. The recombinant plasmid thus obtained was

15 termed pVRC421. Its restriction map is depicted in Figure 11.

2-2-2. Isolation of mutant SP92hpaA::am<sup>R</sup>, whose hpaA gene is disrupted by means of homologous recombination.

20 This example illustrates how the mutant of S. pristinaespiralis SP92 whose hpaA gene is disrupted was constructed.

This mutant was isolated by transforming strain SP92 with the suicide plasmid pVRC421.

25 The protoplasts were prepared and transformed as described previously.

Strain SP92 was cultured, at 30°C for 40 hours, in YEME medium, 34% sucrose, 5 mM MgCl<sub>2</sub>, 0.25%

glycine. The mycelium was protoplasted in the presence of lysozyme, and  $5 \times 1 \mu\text{g}$  of pVRC421 were employed for transforming (by the method using PEG) the protoplasts. After one night for regenerating the protoplasts on

5 R2YE medium, the recombinants were selected by spreading on 3 ml of SNA medium containing  $1,500 \mu\text{g/ml}$  geneticin.

600 clones which were resistant to geneticin were isolated from the 5 transformations which were

10 carried out. These recombinants result from integration by means of simple or double homologous recombination between the hpaA gene carried by the chromosome of strain SP92 and the 6 kb fragment of the suicide plasmid pVRC421. In order to select the recombinants

15 obtained by double crossing over (that is, the clones which no longer contain, in their genome, the pDH5 moiety of plasmid pVRC421), the clones were subcultured on HT7 medium containing  $400 \mu\text{g/ml}$  thiostrepton.

6 clones which were resistant to geneticin but

20 sensitive to thiostrepton were selected. The spores of the recombinants were selected by streaking and growth on HT7 medium containing  $10 \mu\text{g/ml}$  geneticin, and restreaked on the same medium in order to obtain isolated colonies. In order to verify the position of

25 integration of plasmid pVRC421, various Southernblots of the total DNA from the 6 recombinant clones, purified as described by Hopwood et al. 1985, were carried out with hybridization to the 4.8 kb SphI-SphI fragment,

which was used as the probe after having been labelled with [ $\alpha$ - $^{32}$ P]dCTP. The results confirm that these recombinants were obtained by double crossing over between the vector pVRC421 and the chromosome of the SP92 strain, resulting in replacement of the 4.8 kb SphI-SphI fragment, containing the hpaA gene, by a 6 kb SphI-SphI fragment which contains the hpaA gene disrupted by the am<sup>R</sup> gene. One of these mutants was designated SP92hpaA:: $\Omega$ am<sup>R</sup>.

2-2-3. Production of pristinamycins by mutant SP92hpaA:: $\Omega$ am<sup>R</sup>.

This example illustrates how it is established that the mutant of S. pristinaespiralis SP92 whose hpaA gene is disrupted by integration of plasmid pVRC421 no longer produces PI under the standard fermentation conditions.

Mutant SP92hpaA:: $\Omega$ am<sup>R</sup>, and also strain SP92 in the role of control strain, were cultured in liquid production medium. The fermentation was carried out as described in Example 2-1-3, and the pristinamycins were then extracted and assayed as previously described. The results demonstrated that, under the fermentation conditions employed, mutant SP92hpaA:: $\Omega$ am<sup>R</sup> did not produce PI, either at 24, 28 or 32 hrs of fermentation, whereas the control strain produced a quantity of PI which was standard for the 3 time points tested. The quantity of PII produced remained the same for the two strains. Mutant SP92hpaA:: $\Omega$ am<sup>R</sup> is definitely blocked at

a step in the biosynthesis of PI. Complementary fermentation tests were carried out by adding different precursors of PI, separately or together, to the culture in production medium after 16 hours. When 100 mg/l 3-hydroxypicolinic acid are added to the fermentation medium, the mutant then produces PI<sub>1</sub> at a level which is equivalent to the production of PI by the control strain. The fact that mutant SP92<sub>hpaA::Ωam<sup>R</sup></sub> can only be complemented by adding 3-hydroxypicolinic acid demonstrates that the hpaA gene is involved in the synthesis of this precursor.

Construction of this mutant made it possible to produce a strain of S. pristinaespiralis which is mutated as regards its production of PI but which, in the presence of the precursor 3-HPA, is capable of producing PI in a quantity equivalent to that produced by the starting strain. In the same way as in the preceding examples, it can be envisaged that it should be possible, using a mutant of this nature in the presence of novel precursors, to produce new pristinamycins which are modified at the level of the 3-hydroxypicolinic acid residue.

**EXAMPLE 3: Production of compounds of the general formula I by the mutant SP92::pVRC508.**

This example illustrates how the mutant of S. pristinaespiralis SP92 whose papA gene is disrupted by integration of plasmid pVRC508 is able to synthesize new streptogramins in the presence of precursors which

are added to the production medium. These precursors can be derivatives of amino acids and, more particularly, of phenylalanine, but also of  $\alpha$ -ketocarboxylic acids and, more particularly, of phenylpyruvic acid.

The mutant SP92::pVRC508 was cultured in liquid production medium. The fermentation was carried out as follows: 0.5 ml of a suspension of spores from the previously mentioned strain is added, under sterile conditions, to 40 ml of inoculum medium in a 300 ml baffled Erlenmeyer flask. The inoculum medium is made up of 10 g/l corn steep, 15 g/l sucrose, 10 g/l  $(\text{NH}_4)_2\text{SO}_4$ , 1 g/l  $\text{K}_2\text{HPO}_4$ , 3 g/l NaCl, 0.2 g/l  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and 1.25 g/l  $\text{CaCO}_3$ . The pH is adjusted to 6.9 with sodium hydroxide solution before introducing the calcium carbonate. The Erlenmeyer flasks are shaken at 27°C for 44 h on a rotating shaker at a speed of 325 rpm. 2.5 ml of the previous culture, which is 44 h old, are added, under sterile conditions, to 30 ml of production medium in a 300 ml Erlenmeyer flask. The production medium consists of 25 g/l soya flour, 7.5 g/l starch, 22.5 g/l glucose, 3.5 g/l fodder yeast, 0.5 g/l zinc sulphate and 6 g/l calcium carbonate. The pH is adjusted to 6.0 with hydrochloric acid before introducing the calcium carbonate. The Erlenmeyer flasks are shaken at 27°C on a rotating shaker at a speed of 325 rpm. After 16 h, 1 ml of a solution of one of the precursors listed in Table 3 (generally 5 or

10 g/l) is added to the culture. The latter is terminated 8 or 24 h later. The volume of the must is measured immediately, and 2 volumes of mobile phase, consisting of 34% acetonitrile and 66% of a solution of 0.1 M  $\text{KH}_2\text{PO}_4$  (adjusted to pH 2.9 with concentrated  $\text{H}_3\text{PO}_4$ ) are added to it for extracting the pristinamycins. After shaking, the whole is centrifuged and the pristinamycins contained in the supernatant are extracted and purified as described in Example 4. They are also assayed by HPLC by means of injecting 150  $\mu\text{l}$  of the centrifugation supernatant onto a Nucleosil 5-C8 4.6  $\times$  150 mm column, which is eluted with a mixture of 40% acetonitrile and 60% 0.1 M phosphate buffer, pH 2.9. The new I pristinamycins are detected by means of their UV absorbance at 206 nm and, where appropriate, by means of their fluorescence emission (370 nm filter, excitation at 306 nm).



	PRECURSOR	ORIGIN
	phenylalanine	Janssen
✓	4-dimethylaminophenylalanine	Example 33
✓	4-methylaminophenylalanine	Example 34-1
5	4-aminophenylalanine	Janssen 22.794.96
	4-diethylaminophenylalanine	Example 33
	4-ethylaminophenylalanine	Example 33
	4-methylthiophenylalanine	Example 33
	4-methylphenylalanine	J.P.S101-312-4/ Example 33
10	4-methoxyphenylalanine	Janssen 16.975.97
	4-trifluoromethoxyphenylalanine	Example 34-8
	4-methoxycarbonylphenylalanine	Example 33
	4-chlorophenylalanine	Janssen 15.728.14
	4-bromophenylalanine	Janssen 22.779.81
15	4-iodophenylalanine	Bachem F 1675
	4-trifluoromethylphenylalanine	P.C.R. Inc. 12 445-3
	4-tert-butylphenylalanine	Example 35-1

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	4-isopropylphenylalanine	Example 36-1
	3-methylaminophenylalanine	Example 35-3
	3-methoxyphenylalanine	J.P.S. 101-313-2
	3-methylthiophenylalanine	Example 34-11
5	3-fluoro-4-methylphenylalanine	Example 34-5
	4-tert-butylphenylpyruvic acid	Example 33
	4-methylaminophenylpyruvic acid	Example 34-4
	2-naphthylphenylalanine	Bachem F 1865
	4-fluorophenylalanine	Bachem F 1535
10	PRECURSOR	ORIGIN
	3-fluorophenylalanine	Bachem F 2135
	3-ethoxyphenylalanine	Example 37-1
	2,4-dimethylphenylalanine	Example 33
	3,4-dimethylphenylalanine	Example 33
15	3-methylphenylalanine	Example 33
	4-phenylphenylalanine	Example 33
	4-butylphenylalanine	Example 36-3
	2-thienyl-3-alanine	Aldrich 28.728.8
	3-trifluoromethylphenylalanine	Example 33
20	3-hydroxyphenylalanine	Aldrich T 9.039.5
	3-ethylaminophenylalanine	Example 35-6

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	4-aminomethylphenylalanine	Example 33
	4-allylaminophenylalanine	Example 38-2
	4-diallylaminophenylalanine	Example 38-1
	4-allylethylaminophenylalanine	Example 39-4
5	4-ethylpropylaminophenylalanine	Example 39-6
	4-ethylisopropylaminophenylalanine	Example 39-1
	4-ethylmethylcyclopropylamino- phenylalanine	Example 39-8
	4-(1-pyrrolidinyl)phenylalanine	Example 40-1
10	4-O-allyltyrosine	Example 33
	4-O-ethyltyrosine	Example 33
	4-ethylthiophenylalanine	Example 33
	4-ethylthiomethylphenylalanine	Example 41-1
	4-O-(2-chloroethyl)tyrosine	Example 42-1
15	4-acetylphenylalanine	Example 33
	4-ethylphenylalanine	Example 33
	3-dimethylaminophenylalanine	Example 35-10

TABLE III

The following table (TABLE IV) indicates the relative retention times of the new PI which are produced, taking PI<sub>1</sub> as the reference. The absolute

retention times were determined at 25°C in the HPLC system described above; they vary slightly from one injection to another and also in accordance with temperature.

5	Precursor	$t_r$ (relative retention time) of the new PI (Neo PI)		
		Neo PI <sub>A</sub>	Neo PI <sub>B</sub>	Other neo PI
	4-methylaminophenylalanine	0.85		
	4-aminophenylalanine	0.64		
	4-methylthiophenylalanine	1.93	2.73	1.63
	4-methylphenylalanine	1.77	2.65	
10	4-methoxyphenylalanine	1.46		
	4-methoxycarbonylphenylalanine	1.49		
	4-chlorophenylalanine	2.04		
	4-bromophenylalanine	2.16		
15	4-iodophenylalanine	2.42		
	4-trifluoromethylphenylalanine	2.56	3.74	
	4-tert-butylphenylalanine	3.34		

	4-isopropylphenylalanine	2.80		4.35
	3-methylaminophenylalanine	1.15		
	3-methoxyphenylalanine	1.49	2.04	
5	3-fluoro-4-methylphenylalanine	2.93		
	4-tert-butylphenylpyruvic acid	3.34		
	4-methylaminophenylpyruvic acid	0.85		
10	4-ethylaminophenylalanine	0.94		
	4-diethylaminophenylalanine	0.61		
	4-allylaminophenylalanine	1.83		
	4-diallylaminophenylalanine	2.64		
15	4-allylethylaminophenylalanine	2.4		
	4-ethylpropylaminophenylalanine	1.06		
	4-ethylisopropylamino-phenylalanine	0.89		
20	4-ethylmethylcyclopropylaminophenylalanine	1.1		

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5	4-(1-pyrrolidinyl)phenyl- alanine	2.0		
	4-O-trifluoromethyltyrosine	2.42		
	4-O-allyltyrosine	2.62		
	4-O-ethyltyrosine	2.2		
	4-ethylthiophenylalanine	1.96		
10	4-methylthiomethylphenyl- alanine	1.98		
	4-O-(2-chloroethyl)tyrosine	2.45		
	4-acetylphenylalanine	1.61		
	4-ethylphenylalanine	1.86	2.40	
	3-dimethylaminophenyl- alanine	1.49		
15	3-methylthiophenylalanine	1.93		
	3-O-ethyltyrosine	1.78		

TABLE IV

The new PI, with a  $t_R$  of 4.35, for 4-isopropylphenylalanine corresponds to a neo PI<sub>E</sub> which is described in Example 14.

20 The new PI, with a  $t_R$  of 1.63, for 4-methylthiophenylalanine corresponds to a 5 $\gamma$ -hydroxy

neo PI<sub>H</sub>, which is described in Example 5.

The mutant SP92::pVRC508 was otherwise fermented in the presence of 4-dimethylaminophenylalanine. Under these conditions of complementation, mutant SP92::pVRC508 produces a quantity of I<sub>A</sub> pristinamycins which is equivalent to that produced by strain SP92.

**EXAMPLE 4: Preparation of pristinamycin I<sub>B</sub>**  
 [4 $\zeta$ -methylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub>]  
 and of 4 $\zeta$ -amino-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub>  
 4.1: Preparation of pristinamycin I<sub>B</sub> [4 $\zeta$ -methylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub>]

The strain SP92::pVRC508 is cultured in production medium, using 60 Erlenmeyer flasks as described in Example 3, with 1 ml of a 10 g/l aqueous solution of (R,S)-4-methylaminophenylalanine, synthesized as in Example 34-1, being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is successively eluted

with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing pristinamycin I<sub>2</sub> are combined and evaporated. The dry residue is taken up in 6 ml of a mixture of 65% water and 35% acetonitrile and injected onto a semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture of 65% 100 mM phosphate buffer, pH 2.9, and 35% acetonitrile. The fractions containing pristinamycin I<sub>2</sub> are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried on sodium sulphate and then evaporated. 52 mg of pristinamycin I<sub>2</sub> are obtained.

NMR spectrum. <sup>1</sup>H (400 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm, ref. TMS): 0.71 (dd, J=16 and 6 Hz, 1H, 5  $\beta_2$ ), 0.92 (t, J=7.5 Hz, 3H: CH<sub>3</sub> 2  $\gamma$ ), from 1.10 to 1.40 (mt, 2H: 3  $\beta_2$  and 3  $\gamma_2$ ), 1.34 (d, J=7.5 Hz, 3H: CH<sub>3</sub> 1  $\gamma$ ), from 1.50 to 1.85 (mt, 3H: 3  $\gamma_1$  and CH<sub>2</sub> 2  $\beta$ ), 2.03 (mt, 1H, 3  $\beta_1$ ), 2.22 (mt, 1H, 5  $\delta_2$ ), 2.33 (broad d, J=16 Hz, 1H: 5  $\delta_1$ ), 2.40 (d, J=16 Hz, 1H: 5  $\beta_1$ ), 2.82 (mt, 1H: 5  $\epsilon_2$ ), 2.81 (s, 3H: 4 NCH<sub>3</sub> in the para position of the phenyl), 2.90 (dd, J=12 and 4 Hz, 1H: 4  $\beta_2$ ), 3.29 (s, 3H: 4 NCH<sub>3</sub>) from 3.20 to 3.45 and 3.60 (2 mts, 1H each: CH<sub>2</sub> 3  $\delta$ ), 3.40 (t, J=12 Hz, 1H: 4  $\beta_1$ ), 4.57 (dd, J=7 and 8 Hz, 1H, 3  $\alpha$ ), 4.75 (broad dd, J=13 and 7 Hz, 1H: 5  $\epsilon_1$ ), 4.83 (mt, 1H: 2 $\alpha$ ), 4.89 (broad d, J=10 Hz, 1H: 1 $\alpha$ ), 5.24 (dd, J=12 and 4 Hz, 1H: 4  $\alpha$ ), 5.32 (broad d, J=6 Hz, 1H: 5  $\alpha$ ), 5.89 (d, J=9 Hz, 1H: 6  $\alpha$ ), 5.90 (broad q, J = 7.5 Hz, 1H: 1 $\beta$ ), 6.53 (d, J=9 Hz, 1H: NH 2), 6.53



(d, J=8 Hz, 2H: 4 $\epsilon$ ), 7.03 (d, J=8 Hz, 2H: 4 $\delta$ ), from  
7.10 to 7.35 (mt, 5H: aromatic H 6), 7.46 (mt, 2H: 1'H<sub>5</sub>  
and 1'H<sub>4</sub>), 7.85 (dd, J=5.5 and 2 Hz, 1H: 1'H<sub>6</sub>), 8.44  
(d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9 Hz, 1H: NH 6),  
5 11.63 (s, 1H: OH).

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4.2: Preparation of 4'-amino-de(4'-dimethylamino)pristinamycin I<sub>a</sub>

Strain SP92::pVRC508 is cultured in production medium, using 60 Erlenmeyer flasks as described in Example 3, with 1 ml of a 5 g/l aqueous solution of (S)-4-aminophenylalanine being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9 and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I<sub>a</sub> are combined and evaporated. The dry residue is taken up in 6 ml of a mixture consisting of 65% water and 35% acetonitrile and injected onto a semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 65% 100 mM phosphate buffer, pH 2.9, and 35% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is

washed with water, dried over sodium sulphate and then evaporated. 5 mg of 4 $\zeta$ -amino-de(4 $\zeta$ -dimethylamino)pristinamycin I $_a$  are obtained.

NMR spectrum:  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm, ref. TMS): 0.72 (dd,  $J=16$  and  $5.5$  Hz,  $1\text{H}$ ,  $5\beta_2$ ), 0.90 (t,  $J=7.5$  Hz,  $3\text{H}$ :  $\text{CH}_3$   $2\gamma$ ), from 1.10 to 1.40 (mt,  $2\text{H}$ :  $3\beta_2$  and  $3\gamma_2$ ), 1.33 (d,  $J=7.5$  Hz,  $3\text{H}$ :  $\text{CH}_3$   $1\gamma$ ), from 1.50 to 1.85 (mt,  $3\text{H}$ :  $3\gamma_1$  and  $\text{CH}_2$   $2\beta$ ), 2.02 (mt,  $1\text{H}$ ,  $3\beta_1$ ), 2.19 (mt,  $1\text{H}$ ,  $5\delta_2$ ), 2.33 (broad d,  $J=16$  Hz,  $1\text{H}$ :  $5\delta_1$ ), 2.42 (d,  $J=16$  Hz,  $1\text{H}$ :  $5\beta_1$ ), 2.81 (dt,  $J=13$  and  $4$  Hz,  $1\text{H}$ :  $5\epsilon_2$ ), 2.90 (dd,  $J=12$  and  $4$  Hz,  $1\text{H}$ :  $4\beta_2$ ), 3.24 (s,  $3\text{H}$ :  $\text{NCH}_3$   $4$ ), from 3.20 to 3.40 and 3.54 (2 mts,  $1\text{H}$  each:  $\text{CH}_2$   $3\delta$ ), 3.30 (t,  $J=12$  Hz,  $1\text{H}$ :  $4\beta_1$ ), 3.72 (unres.comp.,  $2\text{H}$ :  $\text{ArNH}_2$ ), 4.54 (dd,  $J=7.5$  and  $7$  Hz,  $1\text{H}$ ,  $3\alpha$ ), 4.73 (broad dd,  $J=13$  and  $8$  Hz,  $1\text{H}$ :  $5\epsilon_1$ ), 4.82 (mt,  $1\text{H}$ :  $2\alpha$ ), 4.89 (broad d,  $J=10$  Hz,  $1\text{H}$ :  $1\alpha$ ), 5.22 (dd,  $J=12$  and  $4$  Hz,  $1\text{H}$ :  $4\alpha$ ), 5.32 (broad d,  $J=5.5$  Hz,  $1\text{H}$ :  $5\alpha$ ), 5.89 (mt,  $2\text{H}$ :  $6\alpha$  and  $1\beta$ ), 6.51 (d,  $J=9.5$  Hz,  $1\text{H}$ :  $\text{NH}$   $2$ ), 6.61 (d  $J=8$  Hz,  $2\text{H}$ :  $4\epsilon$ ), 6.98 (d,  $J=8$  Hz,  $2\text{H}$ :  $4\delta$ ), from 7.15 to 7.35 (mt,  $5\text{H}$ : aromatic H  $6$ ), 7.45 (dd,  $J=8.5$  and  $1.5$  Hz,  $1\text{H}$ :  $1'\text{H}_4$ ), 7.48 (dd,  $J=8.5$  and  $4$  Hz,  $1\text{H}$ :  $1'\text{H}_5$ ), 7.82 (dd,  $J=4$  and  $1.5$  Hz,  $1\text{H}$ :  $1'\text{H}_6$ ), 8.43 (d,  $J=10$  Hz,  $1\text{H}$ :  $\text{NH}$   $1$ ), 8.76 (d,  $J=9.5$  Hz,  $1\text{H}$ :  $\text{NH}$   $6$ ), 11.63 (s,  $1\text{H}$ : OH).

Example 5: Preparation of 4 $\zeta$ -methylthio-de(4 $\zeta$ -dimethylamino)pristinamycin I $_A$ , of 4 $\zeta$ -methylthio-de(4 $\zeta$ -dimethylamino)pristinamycin I $_B$  and of 5- $\gamma$ -hydroxy-4 $\zeta$ -methylthio-de(4 $\zeta$ -dimethylamino)pristinamycin I $_B$

5 Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-4-methylthiophenylalanine, synthesized as in Example 33, in 0.1N sodium hydroxide solution being  
10 added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant  
15 is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica  
20 (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I $_A$  are combined and evaporated. 65 mg of dry residue are obtained. This is  
25 taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in two batches onto a semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture

consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed  
 5 with water, dried over sodium sulphate and then evaporated. 45 mg of 4'-methylthio-de(4'-dimethylamino)pristinamycin I<sub>x</sub> are obtained.

NMR spectrum: <sup>1</sup>H (400 MHz, CDCl<sub>3</sub>, δ in ppm, ref. TMS): 0.68 (dd, J=16 and 5.5 Hz, 1H 5 β<sub>2</sub>), 0.93 (t, J=7.5 Hz, 3H: CH<sub>3</sub>, 2 γ), 1.13 (mt, 1H: 3 β<sub>2</sub>), from 1.25 to 1.40 (mt, 1H: 3 γ<sub>2</sub>), 1.33 (d, J=7.5 Hz, 3H: CH<sub>3</sub>, 1 γ), from 1.55 to 1.85 (mt, 3H: 3 γ<sub>1</sub>, and CH<sub>2</sub> 2 β), 2.02 (mt, 1H, 3 β<sub>1</sub>), 2.18 (mt, 1H, 5 δ<sub>2</sub>), 2.38 (broad d, J=16.5 Hz, 1H: 5 δ<sub>1</sub>), 2.46 (s, 3H: SCH<sub>3</sub>), 2.48 (d, J=16 Hz, 1H, 5 β<sub>1</sub>), 2.85 (dt, J=13.5 and 4 Hz, 1H: 5 ε<sub>2</sub>), 3.00 (dd, J=12 and 5 Hz, 1H: 4 β<sub>2</sub>), 3.23 (s, 3H: NCH<sub>3</sub>, 4), 3.37 (t, J=12 Hz, 1H: 4 β<sub>1</sub>), 3.37 and 3.58 (2 mts, 1H each: CH<sub>2</sub> 3 δ), 4.55 (t, J=7.5 Hz, 1H, 3 α), 4.77 (broad dd, J=13.5 and 8 Hz, 1H: 5 ε<sub>1</sub>), 4.86 (mt, 1H: 2α), 4.89 (dd, J=10 and 1.5 Hz, 1H: 1α), 5.30 (broad d, J=5.5 Hz, 1H: 5 α), 5.32 (dd, J=12 and 5 Hz, 1H: 4 α), 5.90 (d, J=9.5 Hz, 1H: 6 α), 5.92 (dq, J=7.5 and 1.5 Hz, 1H: 1β), 6.55 (d, J=9.5 Hz, 1H: NH 2), 7.13 (d, J=8 Hz, 2H: 4δ), from 7.15 to 7.35 (mt, 5H: aromatic H 6), 7.19 (d, J=8 Hz, 2H: 4ε), 7.45 (mt, 2H: 1'H<sub>4</sub> and H<sub>5</sub>), 7.76 (t, J=5 Hz, 1'H<sub>6</sub>), 8.42 (d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9.5 Hz, 1H: NH 6), 11.65 (s, 1H: OH).

Using the fractions derived from the silica column described above which contain the novel derivative of pristinamycin I<sub>H</sub>, 10 mg of 4 $\zeta$ -methylthio-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>H</sub> are isolated by means of semi-preparative column chromatography as described above but bringing the proportion of acetonitrile in the eluent phase to 50%.

NMR spectrum: <sup>1</sup>H (400 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm, ref. TMS): 0.32 (mt, 1H, 5  $\beta_2$ ), 0.93 (t, J=7.5 Hz, 3H: CH<sub>3</sub> 2  $\gamma$ ), from 1.20 to 1.35 (mt, 2H: 3  $\beta_2$  and 3  $\gamma_2$ ), 1.30 (d, J=7.5 Hz, 3H: CH<sub>3</sub> 1  $\gamma$ ), from 1.35 to 2.05 (mt, 9H: 3  $\gamma_1$  - 3  $\beta_1$  - CH<sub>2</sub> 2  $\beta$  - CH<sub>2</sub> 5  $\delta$  - CH<sub>2</sub> 5 $\gamma$  and 5  $\beta_1$ ), 2.44 (dt, J=13.5 and 1.5 Hz, 1H: 5  $\epsilon_2$ ), 2.49 (s, 3H: SCH<sub>3</sub>), 2.99 (dd, J=12 and 5 Hz, 1H: 4  $\beta_2$ ), 3.09 (dd, J=12.5 and 12 Hz, 1H: 4  $\beta_1$ ), 3.54 and 3.64 (2 mts, 1H each: CH<sub>2</sub> 3  $\delta$ ), 4.17 (dd, J=7 and 6 Hz, 1H: 3  $\alpha$ ), 4.49 (broad d, J=13.5 Hz: 1H: 5  $\epsilon_1$ ), from 4.70 to 4.80 (mt, 3H: 2 $\alpha$  - 5  $\alpha$  and 4  $\alpha$ ), 4.84 (dd, J=10 and 1.5 Hz, 1H: 1 $\alpha$ ), 5.51 (d, J=7 Hz, 1H: 6  $\alpha$ ), 5.73 (mt, 1H: 1 $\beta$ ), 6.65 (d, J=9.5 Hz, 1H: NH 2), 7.10 (d, J=8 Hz, 2H: 4 $\delta$ ), 7.22 (d, J=8 Hz, 2H: 4 $\epsilon$ ), from 7.20 to 7.40 (mt, 7H: aromatic H 6 = 1' H<sub>4</sub> and 1' H<sub>5</sub>), 7.87 (d, J=4 Hz, 1H: 1' H<sub>6</sub>), 8.55 (unres.comp., 1H: NH 6), 8.55 (d, J=10 Hz, 1H: NH 1), 11.70 (s, 1H: OH).

Using the fractions derived from the silica column described above which contain the novel derivative of pristinamycin I, 3 mg of 5 $\gamma$ -hydroxy-4 $\zeta$ -methylthio-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>H</sub> are

isolated by carrying out semi-preparative column chromatography as described above and maintaining the proportion of acetonitrile in the eluent phase at 45%.

NMR spectrum:  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm, ref. TMS): a markedly preponderant isomer is observed: the -OH in the 5  $\gamma$  position in an axial position. 0.37 (d mt,  $J=16$  Hz, 1H, 5  $\beta_2$ ), 0.93 (t,  $J=7.5$  Hz, 3H:  $\text{CH}_3$  2  $\gamma$ ), from 1.20 to 1.45 (mt, 2H: 3  $\beta_2$  and 3  $\gamma_2$ ) 1.31 (d,  $J=7.5$  Hz, 3H:  $\text{CH}_3$  1  $\gamma$ ), from 1.40 to 1.85 (mt, 5H: 3  $\gamma_1$  -  $\text{CH}_2$  2  $\beta$  and  $\text{CH}_2$  5  $\delta$ ), 1.98 (mt, 1H, 3  $\beta_1$ ), 2.17 (d,  $J=16$  Hz, 1H: 5  $\beta_1$ ), 2.50 (s, 3H:  $\text{SCH}_3$ ), 2.77 (dt,  $J=13.5$  and 2 Hz, 1H: 5  $\epsilon_2$ ), 2.99 (dd,  $J=12$  and 4 Hz, 1H: 4  $\beta_2$ ), 3.11 (t,  $J=12$  Hz, 1H: 4  $\beta_1$ ), from 3.45 to 3.70 (mt, 2H:  $\text{CH}_2$  3  $\delta$ ), 3.73 (mt, 1H: 5  $\gamma$  in an equatorial position), 4.13 (t,  $J=7$  Hz, 1H, 3  $\alpha$ ), 4.37 (broad d,  $J=13.5$  Hz, 1H: 5  $\epsilon_1$ ), from 4.75 to 4.95 (mt, 3H: 2 $\alpha$ , 4  $\alpha$  and 5  $\alpha$ ), 4.89 (dd,  $J=10$  and 1 Hz, 1H: 1 $\alpha$ ), 5.70 (d,  $J=8$  Hz, 1H: 6  $\alpha$ ), 5.80 (dq,  $J=7.5$  and 1 Hz, 1H: 1 $\beta$ ), 6.37 (d,  $J=5$  Hz, 1H: NH 4), 6.71 (d,  $J=10$  Hz, 1H: NH 2), 7.10 (d,  $J=8$  Hz, 2H: 4 $\delta$ ), 7.22 (d,  $J=8$  Hz, 2H: 4  $\epsilon$ ), from 7.20 to 7.40 (mt, 5H: aromatic H 6), 7.43 (dd,  $J=8.5$  and 1.5 Hz, 1H: 1' $\text{H}_4$ ), 7.46 (dd,  $J=8.5$  and 4 Hz, 1H: 1' $\text{H}_5$ ), 7.89 (dd,  $J=4$  and 1.5 Hz, 1H: 1' $\text{H}_6$ ), 8.55 (d,  $J=10$  Hz, 1H: NH 1), 9.15 (d,  $J=8$  Hz, 1H: NH 6), 11.70 (s, 1H: OH).

EXAMPLE 6: Preparation of 4 $\zeta$ -methyl-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> and of 4 $\zeta$ -methyl-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>B</sub>

Strain SP92::pVRC508 is cultured in

5 production medium, using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution of (R,S)-4-methylphenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from

10 the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed

15 with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10%

20 methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I<sub>A</sub> are combined and evaporated. 49 mg of dry residue are obtained. This residue is taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected, in two

25 batches, onto a semi-preparative Nucleosil 7 $\mu$  C8 10 $\times$ 250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing



the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 44 mg of 4 $\zeta$ -methyl-de(4 $\zeta$ -

5 dimethylamino)pristinamycin I $_A$  are obtained.

NMR spectrum:  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm, ref. TMS): 0.52 (dd,  $J=16$  and  $6$  Hz,  $1\text{H}$ ,  $5\beta_2$ ), 0.93 (t,  $J=7.5$  Hz,  $3\text{H}$ :  $\text{CH}_3$   $2\gamma$ ), 1.15 (mt,  $1\text{H}$ :  $3\beta_2$ ), from 1.20 to 1.40 (mt,  $1\text{H}$ :  $3\gamma_2$ ), 1.35 (d,  $J=7.5$  Hz,  $3\text{H}$ :  $\text{CH}_3$   $1\gamma$ ),  
 10 from 1.50 to 1.85 (mt,  $3\text{H}$ :  $3\gamma_1$  and  $\text{CH}_2$   $2\beta$ ), 2.04 (mt,  $1\text{H}$ ,  $3\beta_1$ ), 2.18 (mt,  $1\text{H}$ ,  $5\delta_2$ ), from 2.25 to 2.45 (mt,  $2\text{H}$ :  $5\delta_1$  and  $5\beta_1$ ), 2.36 (s,  $3\text{H}$ :  $\text{ArCH}_3$ ), 2.83 (dt,  $J=13$  and  $4$  Hz,  $1\text{H}$ :  $5\epsilon_2$ ), 2.99 (dd,  $J=13$  and  $4$  Hz,  $1\text{H}$ :  $4\beta_2$ ), 3.28 (s,  $3\text{H}$ :  $\text{NCH}_3$ ), 3.31 and 3.59 (2 mts,  $1\text{H}$  each:  $\text{CH}_2$   $3\delta$ ), 3.40 (t,  $J=13$  Hz,  $1\text{H}$ :  $4\beta_1$ ), 4.59 (t,  $J=7.5$  Hz,  $1\text{H}$ ,  $3\alpha$ ), 4.74 (broad dd,  $J=13$  and  $7$  Hz,  $1\text{H}$ :  $5\epsilon_1$ ), 4.85 (mt,  $1\text{H}$ :  $2\alpha$ ), 4.89 (broad d,  $J=10$  Hz,  $1\text{H}$ :  $1\alpha$ ), from 5.25 to 5.35 (mt,  $2\text{H}$ :  $5\alpha$  and  $4\alpha$ ), from 5.85 to 5.95 (mt,  $2\text{H}$ :  $6\alpha$  and  $1\beta$ ), 6.52 (d,  $J=9.5$  Hz,  $1\text{H}$ :  $\text{NH } 2$ ),  
 20 7.14 (AB limit,  $J=9$  Hz,  $4\text{H}$ :  $4\delta$  and  $4\epsilon$ ), from 7.15 to 7.35 (mt,  $5\text{H}$ : aromatic H  $6$ ), 7.50 (mt,  $2\text{H}$ :  $1'\text{H}_4$  and  $1'\text{H}_5$ ), 7.81 (dd,  $J=4$  and  $2\text{Hz}$ ,  $1\text{H}$ :  $1'\text{H}_6$ ), 8.41 (d,  $J=10$  Hz,  $1\text{H}$ :  $\text{NH } 1$ ), 8.74 (d,  $J=9$  Hz,  $1\text{H}$ :  $\text{NH } 6$ ), 11.63 (s,  $1\text{H}$ :  $\text{OH}$ ).

25 Using the fractions derived from the silica column described above which contain the new derivative of pristinamycin I $_B$ , 21 mg of 4 $\zeta$ -methyl-de(4 $\zeta$ -dimethylamino)pristinamycin I $_B$  (mass spectrometry:

M+H<sup>+</sup>=810) are isolated by carrying out semi-preparative column chromatography as described above.

**EXAMPLE 7: Preparation of 4}-methoxy-de(4}-dimethylamino)pristinamycin I<sub>1</sub>.**

5                    Strain SP92::pVRC508 is cultured in production medium using 12 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution of (RS)-4-methoxyphenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the end of  
10 40 h of culture, the 0.35 litres of must recovered from the 12 Erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of  
15 dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and  
20 is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I<sub>1</sub> are combined and evaporated. 14 mg of dry residue are obtained. This residue is taken up in 3 ml of a mixture consisting of  
25 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Machery Nagel), which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and

40% acetonitrile. The fractions containing the new  
 pristinamycin are combined and extracted with one  
 volume of dichloromethane. The organic phase is washed  
 with water, dried over sodium sulphate and then  
 5 evaporated. 12 mg of 4'-methoxy-de(4'-  
 dimethylamino)pristinamycin I<sub>x</sub> are obtained.

NMR spectrum: <sup>1</sup>H (400 MHz, CDCl<sub>3</sub>, d in ppm,  
 ref. TMS): 0.63 (dd, J=16 and 5.5 Hz, 1H, 5 β<sub>2</sub>), 0.96  
 (t, J=7.5 Hz, 3H: CH<sub>3</sub> 2 γ), 1.17 (mt, 1H: 3 β<sub>2</sub>), from  
 10 1.30 to 1.45 (mt, 1H: 3 γ<sub>2</sub>), 1.38 (d, J=7.5 Hz, 3H: CH<sub>3</sub>,  
 1 γ) from 1.55 to 1.85 (mt, 3H: 3 γ<sub>1</sub> and CH<sub>2</sub> 2 β), 2.05  
 (mt, 1H, 3 β<sub>1</sub>), 2.20 (mt, 1H, 5 δ<sub>2</sub>), 2.40 (broad d,  
 J=16 Hz, 1H: 5 δ<sub>1</sub>), 2.47 (d, J=16 Hz, 1H: 5 β<sub>1</sub>), 2.88  
 (dt, J=13 and 4 Hz, 1H: 5 ε<sub>2</sub>), 2.99 (dd, J=12.5 and  
 15 5 Hz, 1H: 4 β<sub>2</sub>), 3.30 (s, 3H: NCH<sub>3</sub> 4), 3.32 and 3.60 (2  
 mts, 1H each: CH<sub>2</sub> 3 δ), 3.40 (t, J=12.5 Hz, 1H: 4 β<sub>1</sub>),  
 3.80 (s, 3H: OCH<sub>3</sub>), 4.60 (t, J=7.5 Hz, 1H, 3 α), 4.80  
 (broad dd, J=13 and 8.5 Hz, 1H: 5 ε<sub>1</sub>), 4.88 (mt, 1H:  
 2α), 4.92 (broad d, J=10 Hz, 1H: 1α), 5.31 (dd, J=12.5  
 20 and 5 Hz, 1H: 4 α), 5.34 (broad d, J=5.5 Hz, 1H: 5 α),  
 5.90 (d, J=9 Hz, 1H: 6 α), 5.93 (broad q, J=7.5 Hz, 1H:  
 1β), 6.54 (d, J=9 Hz, 1H: NH 2), 6.87 (d, J=8 Hz, 2H:  
 4ε), 7.16 (d, J=8 Hz, 2H: 4δ), from 7.15 to 7.40 (mt,  
 5H: aromatic H 6), 7.50 (mt, 2H: 1'H<sub>5</sub> and 1'H<sub>4</sub>), 7.80  
 25 (dd, J=4 and 2.5 Hz, 1H: 1'H<sub>6</sub>), 8.43 (d, J=10 Hz, 1H: NH  
 1), 8.78 (d, J=9 Hz, 1H: NH 6), 11.65 (s, 1H:OH).

**EXAMPLE 8: Preparation of 4 $\beta$ -methoxycarbonyl-de(4 $\beta$ -dimethylamino)pristinamycin I<sub>A</sub>.**

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-4-methoxycarbonylphenylalanine, synthesized as in Example 33, being added at 16 h. At the end of 24 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I<sub>A</sub> are combined and evaporated. 14 mg of dry residue are obtained. This residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one

volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 9 mg of 4'-methoxycarbonyl-de(4'-dimethylamino)pristinamycin I<sub>A</sub> are obtained.

- 5 NMR spectrum: <sup>1</sup>H (400 MHz, CDCl<sub>3</sub>, δ in ppm, ref. TMS): 0.70 (dd, J=16 and 6 Hz, 1H, 5 β<sub>2</sub>), 0.93 (t, J=7.5 Hz, 3H: CH<sub>3</sub> 2 γ), 1.08 (mt, 1H: 3 β<sub>2</sub>), from 1.30 to 1.40 (mt, 1H: 3 γ<sub>2</sub>), 1.33 (d, J=7.5 Hz, 3H: CH<sub>3</sub> 1 γ) from 1.55 to 1.85 (mt, 3H: 3 γ<sub>1</sub> and CH<sub>2</sub> 2 β), 2.02 (mt, 10 1H, 3 β<sub>1</sub>), 2.13 (mt, 1H, 5 δ<sub>2</sub>), 2.40 (broad d, J=16.5 Hz, 1H: 5 δ<sub>1</sub>), 2.48 (d, J=16 Hz, 1H, 5 β<sub>1</sub>), 2.89 (dt, J=14.5 and 4.5 Hz, 1H: 5 ε<sub>2</sub>), 3.10 (dd, J=13.5 and 6 Hz, 1H: 4 β<sub>2</sub>), 3.24 (s, 3H: NCH<sub>3</sub> 4), 3.38 and 3.61 (2 mts, 1H each: CH<sub>2</sub> 3 δ), 3.47 (t, J=13.5 Hz, 1H: 4 β<sub>1</sub>), 15 3.96 (s, 3H: COOCH<sub>3</sub>), 4.55 (t, J=7.5 Hz, 1H, 3 α), 4.78 (broad dd, J=14.5 and 8 Hz, 1H: 5 ε<sub>1</sub>), 4.86 (mt, 1H: 2α), 4.89 (broad d, J=10 Hz, 1H: 1α), 5.33 (broad d, J=6 Hz, 1H: 5 α), 5.42 (dd, J=13.5 and 6 Hz, 1H: 4 α), 5.92 (d, (J=9.5 Hz) and mt, 1H each: 6 α and 1β respectively), 6.52 (d, J=10 Hz, 1H: NH 2), from 7.15 20 to 7.35 (mt, 5H: aromatic H 6), 7.28 (d, J=8 Hz, 2H: 4δ), 7.43 (dd, J=9 and 1.5 Hz, 1H: 1'H<sub>4</sub>), 7.47 (dd, J=9 and 5 Hz, 1H: 1'H<sub>5</sub>), 7.66 (d, J=5 and 1.5 Hz, 1H: 1'H<sub>6</sub>), 7.98 (d, J=8 Hz, 2H: 4ε), 8.38 (d, J=10 Hz, 1H: 25 NH 1), 8.76 (d, J=9.5 Hz, 1H: NH 6), 11.70 (s, 1H: OH).

**EXAMPLE 9: Preparation of 4 $\beta$ -chloro-de(4 $\beta$ -dimethylamino)pristinamycin I $_A$ .**

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-4-chlorophenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I $_A$  are combined and evaporated. The dry residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is

washed with water, dried over sodium sulphate and then evaporated. 1 mg of 4 $\zeta$ -chloro-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> is obtained.

NMR spectrum: <sup>1</sup>H (400 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm,  
 5 ref. TMS): 0.93 (t, J=7.5 Hz, 3H: CH<sub>3</sub> 2  $\gamma$ ), 0.95 (dd, J=16 and 5 Hz, 1H, 5  $\beta_2$ ), 1.09 (mt, 1H: 3  $\beta_2$ ), from 1.20 to 1.40 (mt, 1H: 3  $\gamma_2$ ), 1.35 (d, J=7.5 Hz, 3H: CH<sub>3</sub> 1  $\gamma$ ) from 1.50 to 1.85 (mt, 3H: 3  $\gamma_1$  and CH<sub>2</sub> 2  $\beta$ ), 2.02 (mt, 1H, 3  $\beta_1$ ), 2.17 (mt, 1H, 5  $\delta_2$ ), 2.43 (broad d, J=16 Hz,  
 10 1H: 5  $\delta_1$ ), 2.59 (d, J=16 Hz, 1H: 5  $\beta_1$ ), 2.90 (dt, J=13.5 and 4 Hz, 1H: 5  $\epsilon_2$ ), 3.04 (dd, J=13 and 6 Hz, 1H: 4  $\beta_2$ ), 3.21 (s, 3H: 4 NCH<sub>3</sub>), 3.36 (t, J=13 Hz, 1H: 4  $\beta_1$ ), 3.39 and 3.59 (2 mts, 1H each: CH<sub>2</sub> 3  $\delta$ ), 4.53 (t, J=7.5 Hz, 1H, 3  $\alpha$ ), 4.76 (broad dd, J=13.5 and 8 Hz, 1H: 5  $\epsilon_1$ ),  
 15 4.86 (mt, 1H: 2 $\alpha$ ), 4.87 (broad d, J=10 Hz, 1H: 1 $\alpha$ ), 5.38 (mt, 2H: 5  $\alpha$  and 4  $\alpha$ ), 5.93 (mt, 2H: 6  $\alpha$  and 1 $\beta$ ), 6.52 (d, J=10 Hz, 1H: NH 2), 7.12 (d, J=8 Hz, 2H: 4 $\delta$ ) from 7.15 to 7.35 (mt, 7H: aromatic H 6 and 4 $\epsilon$ ), 7.38 (dd, J=9 and 4.5 Hz, 1H: 1' $H_5$ ), 7.43 (broad d, J=9 Hz,  
 20 1H: 1' $H_4$ ), 7.68 (dd, J=4.5 and 1 Hz, 1H: 1' $H_6$ ), 8.36 (d, J=10 Hz, 1H: NH 1), 8.75 (d, J=9 Hz, 1H: NH 6), 11.65 (s, 1H: OH).

EXAMPLE 10: Preparation of 4 $\zeta$ -bromo-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> and of 4 $\zeta$ -bromo-de(4 $\zeta$ -  
 25 dimethylamino)pristinamycin I<sub>B</sub>.

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution

of (R,S)-4-bromophenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I<sub>A</sub> are combined and evaporated. The dry residue is taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in two batches onto a semi-preparative Nucleosil 7 $\mu$  C8 10 $\times$ 250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 6 mg of 4 $\beta$ -bromo-de(4 $\beta$ -dimethylamino)pristinamycin I<sub>A</sub> are obtained.

NMR spectrum: <sup>1</sup>H (400 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm, ref. TMS): 0.93 (J=7.5 Hz, 3H: CH<sub>3</sub>, 2  $\gamma$ ), 0.95 (dd, J=16



and 5 Hz, 1H, 5  $\beta_2$ ), 1.10 (mt, 1H: 3  $\beta_2$ ), 1.35 (d, J=7.5 Hz, 3H: CH<sub>3</sub>, 1  $\gamma$ ) 1.36 (mt, 1H: 3  $\gamma_2$ ), from 1.50 to 1.85 (mt, 3H, 3  $\gamma_1$  and CH<sub>2</sub>, 2  $\beta$ ), 2.02 (mt, 1H, 3  $\beta_1$ ), 2.18 (mt, 1H: 5  $\delta_2$ ), 2.43 (broad d, J=16 Hz, 1H: 5  $\delta_1$ ), 2.59 (d, J=16 Hz, 1H: 5  $\beta_1$ ), 2.90 (dt, J=13 and 4 Hz, 1H: 5  $\epsilon_2$ ), 3.02 (dd, J=13 and 5.5 Hz, 1H: 4  $\beta_2$ ), 3.21 (s, 3H: 4 NCH<sub>3</sub>), 3.33 (dd, J=13-11 Hz, 1H: 4  $\beta_1$ ), 3.39 and 3.59 (2 mts, 1H each: CH<sub>2</sub>, 3  $\delta$ ), 4.53 (t, J=7.5 Hz, 1H, 3  $\alpha$ ), 4.76 (broad dd, J=13 and 7 Hz, 1H: 5  $\epsilon_1$ ), 4.86 (mt, 1H, 2 $\alpha$ ), 4.89 (d broad, J=10 Hz, 1H: 1 $\alpha$ ), 5.37 (broad d, J=5 Hz, 1H: 5  $\alpha$ ), (dd, J=11 and 5.5 Hz, 1H: 4  $\alpha$ ), 5.92 (mt, 2H: 6  $\alpha$  and 1 $\beta$ ), 6.56 (d, J=9.5 Hz, 1H: NH 2), 7.08 (d, J=8 Hz, 2H: 4 $\delta$ ), from 7.15 to 7.35 (mt, 5H: aromatic H 6), 7.40 (mt, 4H: 1'H<sub>4</sub> - 1'H<sub>5</sub> and 4 $\epsilon$ ), 7.70 (broad d, J=5 Hz, 1H: 1'H<sub>6</sub>), 8.40 (d, J=10 Hz, 1H: NH 1), 8.77 (d, J=9 Hz, 1H: NH 6), 11.68 (s, 1H: OH).

Using the fractions derived from the silica column described above which contain the new derivative of pristinamycin I<sub>R</sub>, 3 mg of 4 $\zeta$ -bromo-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>R</sub> (mass spectrometry: M+H<sup>+</sup>=874) are isolated by carrying out semi-preparative column chromatography as described above.

EXAMPLE 11: Preparation of 4 $\zeta$ -iodo-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> and of 4 $\zeta$ -iodo-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>R</sub>.

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution

of (RS)-4-iodophenylalanine in sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried on sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I<sub>a</sub> are combined and evaporated. The dry residue is taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in two batches onto a semi-preparative Nucleosil 7 $\mu$  C8 10 $\times$ 250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 12 mg of 4 $\zeta$ -iodo-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>a</sub> are obtained.

NMR spectrum:  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm, ref. TMS): 0.93 (J=7.5 Hz, 3H:  $\text{CH}_3$ , 2  $\gamma$ ), 0.95 (dd, J=16

and 5.5 Hz, 1H: 5  $\beta_2$ ), 1.10 (mt, 1H: 3  $\beta_2$ ), 1.35 (d, J=7.5 Hz, 3H: CH<sub>3</sub> 1  $\gamma$ ), 1.38 (mt, 1H: 3  $\gamma_2$ ), from 1.55 to 1.85 (mt, 3H, 3  $\gamma_1$  and CH<sub>2</sub> 2  $\beta$ ), 2.02 (mt, 1H, 3  $\beta_1$ ), 2.17 (mt, 1H: 5  $\delta_2$ ); 2.43 (broad d, J=16.5 Hz, 1H: 5  $\delta_1$ ), 2.60 (d, J=16 Hz, 1H: 5  $\beta_1$ ), 2.89 (dt, J=14 and 4.5 Hz, 1H: 5  $\epsilon_2$ ), 3.02 (dd, J=13 and 5.5 Hz, 1H: 4  $\beta_2$ ), 3.21 (s, 3H: NCH<sub>3</sub> 4), 3.31 (dd, J=13 and 11 Hz, 1H: 4  $\beta_1$ ), 3.39 and 3.59 (2 mts, 1H each: CH<sub>2</sub> 3  $\delta$ ), 4.53 (t, J=7.5 Hz, 1H, 3  $\alpha$ ), 4.75 (broad dd, J=14 and 8 Hz, 1H: 5  $\epsilon_1$ ), 4.83 (mt, 1H: 2 $\alpha$ ), 4.88 (broad d, J=10 Hz, 1H: 1 $\alpha$ ), 5.37 (broad d, J=5.5 Hz, 1H: 5  $\alpha$ ), 5.39 (dd, J=11 and 5.5 Hz, 1H: 4  $\alpha$ ), 5.92 (mt, 2H: 6  $\alpha$  and 1 $\beta$ ), 6.54 (d, J=9.5 Hz, 1H: NH 2), 6.94 (d, J=7.5 Hz, 2H: 4 $\delta$ ), from 7.15 to 7.50 (mt, 5H: aromatic H 6), 7.36 (dd, J=9 and 4 Hz, 1H: 1' $H_5$ ), 7.43 (broad d, J=9 Hz, 1H: 1' $H_4$ ), 7.62 (d, J=7.5 Hz, 2H: 4 $\epsilon$ ), 7.68 (d, J=4 Hz, 1H: 1' $H_6$ ), 8.38 (d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9 Hz, 1H: NH 6), 11.60 (s, 1H: OH).

Using the fractions derived from the silica column described above which contain the new derivative of pristinamycin I<sub>B</sub>, 6 mg of 4 $\zeta$ -iodo-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>B</sub> (mass spectrometry: M+H<sup>+</sup>=922) are isolated by carrying out semi-preparative column chromatography as described above.

**EXAMPLE 12** Preparation of 4 $\zeta$ -trifluoromethyl-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> and of 4 $\zeta$ -trifluoromethyl-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>B</sub>.

Strain SP92::pVRC508 is cultured in

production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution of (S)-4-trifluoromethylphenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried on sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I<sub>A</sub> are combined and evaporated. The dry residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 5 mg of 4 $\beta$ -trifluoromethyl-de(4 $\beta$ -dimethylamino)pristinamycin I<sub>A</sub> are obtained.

NMR spectrum:  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm,  
 ref. TMS): 0.86 (dd,  $J=16$  and  $5.5$  Hz,  $1\text{H}$ ,  $5\beta_2$ ), 0.91  
 (t,  $J=7.5$  Hz,  $3\text{H}$ :  $\text{CH}_3$   $2\gamma$ ), 1.13 (mt,  $1\text{H}$ :  $3\beta_2$ ), 1.31 (d,  
 $J=7.5$  Hz,  $3\text{H}$ :  $\text{CH}_3$   $1\gamma$ ) 1.42 (mt,  $1\text{H}$ :  $3\gamma_2$ ), from 1.55 to  
 5 1.80 (mt,  $3\text{H}$ :  $3\gamma_1$  and  $\text{CH}_2$   $2\beta$ ), 2.02 (mt,  $1\text{H}$ ,  $3\beta_1$ ),  
 2.15 (mt,  $1\text{H}$ ,  $5\delta_2$ ), 2.40 (broad d,  $J=16.5$  Hz,  $1\text{H}$ :  $5\delta_1$ ),  
 $2.55$  (d,  $J=16$  Hz,  $1\text{H}$ :  $5\beta_1$ ),  $2.88$  (dt,  $J=14$  and  
 $4$  Hz,  $1\text{H}$ :  $5\epsilon_2$ ),  $3.18$  (s,  $3\text{H}$ :  $\text{NCH}_3$   $4$ ),  $3.20$  and  $3.31$   
 (2 dd, respectively  $J=13$  and  $6$  Hz and  $J=13$  and  $10$  Hz,  
 10  $1\text{H}$  each:  $4\beta_2$  and  $4\beta_1$ ),  $3.42$  and  $3.60$  (2 mts,  $1\text{H}$  each:  
 $\text{CH}_2$   $3\delta$ ),  $4.50$  (t,  $J=7.5$  Hz,  $1\text{H}$ ,  $3\alpha$ ),  $4.73$  (broad dd,  
 $J=14$  and  $7.5$  Hz,  $1\text{H}$ :  $5\epsilon_1$ ),  $4.83$  (mt,  $1\text{H}$ :  $2\alpha$ ),  $4.91$   
 (broad d,  $J=10$  Hz,  $1\text{H}$ :  $1\alpha$ ),  $5.40$  (broad d,  $J=5.5$  Hz,  
 $1\text{H}$ :  $5\alpha$ ),  $5.55$  (dd,  $J=10$  and  $6$  Hz,  $1\text{H}$ :  $4\alpha$ ),  $5.87$  (d,  
 15  $J=9.5$  Hz,  $1\text{H}$ :  $6\alpha$ ),  $5.90$  (broad q,  $J=7.5$  Hz,  $1\text{H}$ :  $1\beta$ ),  
 $6.68$  (d,  $J=9.5$  Hz,  $1\text{H}$ :  $\text{NH}$   $2$ ), from  $7.15$  to  $7.40$  (mt,  
 $9\text{H}$ :  $4\delta$ -aromatic  $\text{H}$   $6 - 1'\text{H}_5$  and  $1'\text{H}_4$ ),  $7.52$  (d,  $J=8$  Hz,  
 $2\text{H}$ :  $4\epsilon$ ),  $7.68$  (d,  $J=4$  and  $1.5$  Hz,  $1\text{H}$ :  $1'\text{H}_6$ ),  $8.43$  (d,  
 $J=10$  Hz,  $1\text{H}$ :  $\text{NH}$   $1$ ),  $8.76$  (d,  $J=9.5$  Hz,  $1\text{H}$ :  $\text{NH}$   $6$ ),  $11.70$   
 20 (s,  $1\text{H}$ :  $\text{OH}$ ).

Using the fractions derived from the silica  
 column described above which contain the new derivative  
 of pristinamycin  $\text{I}_\text{H}$ ,  $4$  mg of  $\zeta$ -trifluoromethyl-de( $4\zeta$ -  
 dimethylamino)pristinamycin  $\text{I}_\text{H}$  (mass spectrometry:  
 25  $\text{M}+\text{H}^+=864$ ) are isolated by carrying out semi-preparative  
 column chromatography as described above.

**EXAMPLE 13: Preparation of 4 $\zeta$ -tert-butyl-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>1</sub>.**

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution of (R,S)-4-tert-butylphenylalanine, synthesized as in Example 35-1, in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyers are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried on sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I<sub>1</sub> are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in 2 batches onto a semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with

one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 30 mg of 4 $\zeta$ -tert-butyl-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>a</sub> are obtained.

- 5 NMR spectrum:  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm, ref. TMS, ref. TMS): 0.21 (dd,  $J=16$  and 5.5 Hz, 1H, 5  $\beta_2$ ), 0.91 (t,  $J=7.5$  Hz, 3H:  $\text{CH}_3$  2  $\gamma$ ), 1.17 (mt, 1H: 3  $\beta_2$ ), from 1.20 to 1.40 (mt, 1H: 3  $\gamma_2$ ), 1.33 (s, 9H:  $\text{CH}_3$  of tert-butyl), 1.35 (d,  $J=7.5$  Hz, 3H:  $\text{CH}_3$  1  $\gamma$ ), from 10 1.50 to 1.85 (mt, 3H: 3  $\gamma_1$  and  $\text{CH}_2$  2  $\beta$ ), 2.04 (mt, 1H, 3  $\beta_1$ ), 2.13 (mt, 1H, 5  $\delta_2$ ), 2.30 (mt, 2H: 5  $\delta_1$  and 5  $\beta_1$ ), 2.80 (dt,  $J=13$  and 4 Hz, 1H: 5  $\epsilon_2$ ), 3.00 (dd,  $J=12$  and 4 Hz, 1H: 4  $\beta_2$ ), 3.29 (s, 3H:  $\text{NCH}_3$ ), 3.31 and 3.59 (2 mts, 1H each:  $\text{CH}_2$  3  $\delta$ ), 3.40 (t,  $J=12$  Hz, 1H: 4  $\beta_1$ ), 15 4.57 (t,  $J=7.5$  Hz, 1H, 3  $\alpha$ ), 4.74 (broad dd,  $J=13$  and 7 Hz, 1H: 5  $\epsilon_1$ ), 4.85 (mt, 1H: 2 $\alpha$ ), 4.90 (broad d,  $J=10$  Hz, 1H: 1 $\alpha$ ), 5.21 (broad d,  $J=5.5$  Hz, 1H: 5  $\alpha$ ), 5.25 (dd,  $J=12$  and 4 Hz, 1H: 4  $\alpha$ ), 5.87 (d,  $J=9$  Hz, 1H: 6  $\alpha$ ), 5.92 (broad q,  $J=7.5$  Hz, 1H: 1 [lacuna] 1H: 1' $\text{H}_6$ ), 20 8.45 (d,  $J=10$  Hz, 1H: NH 1), 8.74 (d,  $J=9$  Hz, 1H: NH 6), 11.65 (s, 1H: OH).

**EXAMPLE 14:** Preparation of 4 $\beta$ -isopropyl-de(4 $\beta$ -dimethylamino)pristinamycin I<sub>A</sub> and of 4 $\beta$ -isopropyl-de(4 $\beta$ -dimethylamino)pristinamycin I<sub>B</sub>.

Strain SP92::pVRC508 is cultured in

5 production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-4-isopropylphenylalanine, synthesized as in Example 36-1, in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the

10 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of

15 dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and

20 eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I<sub>A</sub> are combined and evaporated. 61 mg of the dry residue are obtained. This residue is taken up in 9 ml of a mixture consisting of

25 60% water and 40% acetonitrile and injected in 3 batches onto a semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer,



pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then  
 5 evaporated. 51 mg of 4 $\zeta$ -isopropyl-de(4 $\zeta$ -dimethylamino)pristinamycin I $_A$  are obtained.

NMR spectrum:  $^1\text{H}$  (250 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm, ref. TMS, ref. TMS): 0.31 (dd,  $J=16$  and  $5.5$  Hz,  $1\text{H}$ ,  $5\beta_2$ ), 0.91 (t,  $J=7.5$  Hz,  $3\text{H}$ :  $\text{CH}_3$  2  $\gamma$ ), from 1.00 to 1.45  
 10 (mt,  $2\text{H}$ : 3  $\beta_2$  and 3  $\gamma_2$ ), 1.25 (d,  $J=7.5$  Hz,  $6\text{H}$ :  $\text{CH}_3$  of isopropyl), 1.35 (d,  $J=7.5$  Hz,  $3\text{H}$ :  $\text{CH}_3$  1  $\gamma$ ), from 1.50 to 1.85 (mt,  $3\text{H}$ : 3  $\gamma_1$  and  $\text{CH}_2$  2  $\beta$ ), from 1.95 to 2.20 (mt,  $2\text{H}$ , 3  $\beta_1$  and 5  $\delta_2$ ), 2.30 (mt,  $2\text{H}$ : 5  $\delta_1$  and 5  $\beta_1$ ), 2.80 (dt,  $J=13$  and  $4$  Hz,  $1\text{H}$ : 5  $\epsilon_2$ ), 2.88 (mt,  $1\text{H}$ :  $\text{CH}$  of  
 15 isopropyl), 2.98 (dd,  $J=12$  and  $4$  Hz,  $1\text{H}$ : 4  $\beta_2$ ), 3.30 (s,  $3\text{H}$ :  $\text{NCH}_3$  4), 3.32 and 3.55 (2 mts,  $1\text{H}$  each:  $\text{CH}_2$  3  $\delta$ ), 3.38 (t,  $J=12$  Hz,  $1\text{H}$ : 4  $\beta_1$ ), 4.55 (t,  $J=7.5$  Hz,  $1\text{H}$ , 3  $\alpha$ ), 4.72 (broad dd,  $J=13$  and  $7$  Hz,  $1\text{H}$ : 5  $\epsilon_1$ ), 4.85 (mt,  $1\text{H}$ :  $2\alpha$ ), 4.88 (broad d,  $J=10$  Hz,  $1\text{H}$ :  $1\alpha$ ), 5.21 (broad  
 20 d,  $J=5.5$  Hz,  $1\text{H}$ :  $5\alpha$ ), 5.25 (dd,  $J=12$  and  $4$  Hz,  $1\text{H}$ : 4  $\alpha$ ), 5.87 (d,  $J=9$  Hz,  $1\text{H}$ : 6  $\alpha$ ), 5.90 (broad q,  $J=7.5$  Hz,  $1\text{H}$ :  $1\beta$ ), 6.50 (d,  $J=9.5$  Hz,  $1\text{H}$ :  $\text{NH}$  2), from 7.05 to 7.35 (mt,  $9\text{H}$ : aromatic H 6 - 4 $\epsilon$  and 4 $\delta$ ), 7.50 (mt,  $2\text{H}$ :  $1'\text{H}_5$  and  $1'\text{H}_4$ ), 7.86 (dd,  $J=4$  and  $1.5$  Hz,  $1\text{H}$ :  
 25  $1'\text{H}_6$ ), 8.40 (d,  $J=10$  Hz,  $1\text{H}$ :  $\text{NH}$  1), 8.72 (d,  $J=9$  Hz,  $1\text{H}$ :  $\text{NH}$  6), 11.60 (s,  $1\text{H}$ :  $\text{OH}$ ).

Using the same fractions derived from the silica column described above, which fractions also

contain the new derivative of pristinamycin I<sub>x</sub>, 5 mg of  $\zeta$ -isopropyl-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>x</sub> are isolated by carrying out semi-preparative column chromatography as described above.

5 NMR spectrum: <sup>1</sup>H (400 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm, ref. TMS): 0.20 (mt, 1H, 5  $\beta_2$ ), 0.92 (t, J=7.5 Hz, 3H: CH<sub>3</sub> 2  $\gamma$ ), from 1.15 to 1.40 (mt, 2H: 3  $\beta_2$  and 3  $\gamma_2$ ), 1.24 (d, J=7.5 Hz, 6H: CH<sub>3</sub> of isopropyl), 1.34 (d, J=7.5 Hz, 3H: CH<sub>3</sub> 1  $\gamma$ ), from 1.35 to 2.05 (mt, 9H: 3  $\gamma_1$   
10 - 3  $\beta_1$  - CH<sub>2</sub> 2  $\beta$  - CH<sub>2</sub> 5  $\delta$  - CH<sub>2</sub> 5  $\gamma$  and 5  $\beta_1$ ), 2.45 (dt, J=13 and 1.5 Hz, 1H: 5 $\epsilon_2$ ), 2.89 (mt, 1H: ArCH), 3.09 (dd, J=14 and 7 Hz, 1H: 4  $\beta_2$ ), 3.17 (s, 3H: NCH<sub>3</sub> 4), 3.25 (dd, J=14 and 9 Hz, 1H: 4  $\beta_1$ ), 3.32 and 3.52 (2 mts, 1H each: CH<sub>2</sub> 3  $\delta$ ), 4.55 (mt, 2H: 3  $\alpha$  and 5  $\epsilon_1$ ),  
15 4.80 (mt, 1H: 2 $\alpha$ ), 4.89 (dd, J=10 and 1.5 Hz, 1H: 1 $\alpha$ ), 4.90 (mt, 1H: 5  $\alpha$ ), 5.35 (dd, J=9 and 7 Hz, 1H: 4  $\alpha$ ), 5.60 (d, J=8 Hz, 1H: 6  $\alpha$ ), 5.89 (dq, J=7.5 and 1.5 Hz, 1H: 1 $\beta$ ), 6.65 (d, J=9.5 Hz, 1H: NH 2), 7.08 (d, J=8 Hz, 2H: 4 $\delta$ ), 7.14 (d, J=8 Hz, 2H: 4 $\epsilon$ ), from 7.20 to 7.40  
20 (mt, 7H: aromatic H 6 - 1'H<sub>4</sub> and 1'H<sub>5</sub>), 7.77 (broad d, J=4 Hz, 1H: 1'H<sub>6</sub>), 8.46 (d, J=10 Hz, 1H: NH 1), 8.48 (d, J=8 Hz, 1H: NH 6), 11.70 (s, 1H: OH).

EXAMPLE 15: Preparation of 4 $\epsilon$ -methylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>x</sub>.

25 Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-3-methylaminophenylalanine, synthesized as in

Example 35-3, in water being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% of 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I<sub>a</sub> are combined and evaporated. 19 mg of dry residue are obtained. This residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 $\mu$  C8 10 $\times$ 250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 8 mg of 4 $\epsilon$ -methylamino-de(4 $\gamma$ -dimethylamino)pristinamycin I<sub>a</sub> are obtained.

NMR spectrum:  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm,

ref. TMS): 0.93 (t,  $J=7.5$  Hz, 3H:  $\text{CH}_3$  2  $\gamma$ ), 1.00 (dd,  $J=16$  and 6 Hz, 1H, 5  $\beta_2$ ), 1.17 (mt, 1H: 3  $\beta_2$ ), from 1.25 to 1.40 (mt, 2H: 3  $\gamma_2$ ), 1.35 (d,  $J=7.5$  Hz, 3H:  $\text{CH}_3$  1  $\gamma$ ), from 1.55 to 1.80 (mt, 3H: 3  $\gamma_1$  and  $\text{CH}_2$  2  $\beta$ ), 2.03 (mt, 1H, 3  $\beta_1$ ), 2.23 (mt, 1H, 5  $\delta_2$ ), 2.39 (broad d,  $J=16$  Hz, 1H: 5  $\delta_1$ ), 2.52 (d,  $J=16$  Hz, 1H: 5  $\beta_1$ ), 2.78 (s, 3H:  $\text{ArNCH}_3$  4), 2.85 (dt,  $J=13$  and 4 Hz, 1H: 5  $\epsilon_2$ ), 2.99 (dd,  $J=13$  and 4.5 Hz, 1H: 4  $\beta_2$ ), 3.23 (s, 3H:  $\text{NCH}_3$  4), 3.25 (t,  $J=13$  Hz, 1H: 4  $\beta_1$ ), 3.38 and 3.58 (2 mts, 1H each:  $\text{CH}_2$  3  $\delta$ ), 4.05 (unres. comp., 1H:  $\text{ArNH}$ ), 4.58 (dd,  $J=6.5$  and 7.5 Hz, 1H, 3  $\alpha$ ), 4.76 (broad dd,  $J=13$  and 8 Hz, 1H: 5  $\epsilon_1$ ), 4.85 (mt, 1H: 2 $\alpha$ ), 4.87 (broad d,  $J=10$  Hz, 1H: 1 $\alpha$ ), 5.35 (dd,  $J=13$  and 4.5 Hz, 1H: 4  $\alpha$ ), 5.38 (broad d,  $J=6$  Hz, 1H: 5  $\alpha$ ), 5.90 (d,  $J=9.5$  Hz, 1H: 6  $\alpha$ ), 5.91 (mt, 1H: 1 $\beta$ ), 6.36 (broad s, 1H: H 2 of the aromatic moiety at position 4), from 6.45 to 6.55 (mt, 2H: H4 and H6 of the aromatic moiety in position 4), 6.53 (d,  $J=10$  Hz, 1H: NH 2), 7.12 (t,  $J=8$  Hz, 1H: H 5 of the aromatic moiety in position 4), from 7.15 to 7.45 (mt, 5H: aromatic H 6), 7.35 (mt, 2H: 1'  $\text{H}_4$  and 1'  $\text{H}_5$ ), 7.75 (t,  $J=3$  Hz, 1H: 1'  $\text{H}_6$ ), 8.40 (d,  $J=10$  Hz, 1H: NH 1), 8.78 (d,  $J=9.5$  Hz, 1H: NH 6), 11.60 (s, 1H: OH).

EXAMPLE 16: Preparation of 4 $\epsilon$ -methoxy-de(4 $\zeta$ -dimethylamino)pristinamycin I $_A$  and of 4 $\epsilon$ -methoxy-de(4 $\zeta$ -dimethylamino)pristinamycin I $_B$ .

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution

of (S)-3-methoxyphenylalanine in 0.1N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I<sub>a</sub> are combined and evaporated. 41 mg of dry residue are obtained. This residue is taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in 2 batches onto a semi-preparative Nucleosil 7 $\mu$  C8 10 $\times$ 250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 28 mg of 4 $\epsilon$ -methoxy-de(4 $\gamma$ -dimethylamino)pristinamycin I<sub>a</sub> are obtained.

NMR spectrum:  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm,

ref. TMS): 0.52 (dd,  $J=16$  and  $5.5$  Hz,  $1H$ ,  $5\beta_2$ ), 0.90  
 (t,  $J=7.5$  Hz,  $3H$ :  $CH_3$  2  $\gamma$ ), from 1.10 to 1.34 (mt,  $2H$ : 3  
 $\beta_2$  and 3  $\gamma_2$ ), 1.34 (d,  $J=7.5$  Hz,  $3H$ :  $CH_3$  1 $\gamma$ ), from 1.50  
 to 1.80 (mt,  $3H$ : 3  $\gamma_1$  and  $CH_2$  2  $\beta$ ), 2.40 (mt,  $1H$ , 3  $\beta_1$ ),  
 5 2.20 (mt,  $1H$ , 5  $\delta_2$ ), 2.35 (broad d,  $J=16$  Hz,  $1H$ : 5  $\delta_1$ ),  
 2.38 (d,  $J=16$  Hz,  $1H$ : 5  $\beta_1$ ), 2.83 (dt,  $J=13$  and  $4$  Hz,  
 $1H$ : 5  $\epsilon_2$ ), 2.97 (dd,  $J=12$  and  $4$  Hz,  $1H$ : 4  $\beta_2$ ), 3.28 (s,  
 $3H$ :  $NCH_3$  4), 3.28 and 3.56 (2 mts,  $1H$  each:  $CH_2$  3  $\delta$ ),  
 3.40 (t,  $J=12$  Hz,  $1H$ : 4  $\beta_1$ ), 3.80 (s,  $3H$ :  $OCH_3$ ), 4.58  
 10 (t,  $J=7.5$  Hz,  $1H$ , 3  $\alpha$ ), 4.76 (broad dd,  $J=13$  and  $8$  Hz,  
 $1H$ : 5  $\epsilon_1$ ), 4.85 (mt,  $1H$ : 2 $\alpha$ ), 4.90 (broad d,  $J=10$  Hz,  
 $1H$ : 1 $\alpha$ ): 5.27 (dd,  $J=12$  and  $4$  Hz,  $1H$ : 4  $\alpha$ ), 5.30 (broad  
 d,  $J=5.5$  Hz,  $1H$ : 5  $\alpha$ ), 5.89 (d,  $J=9.5$  Hz,  $1H$ : 6  $\alpha$ ),  
 5.91 (broad q,  $J=7.5$  Hz,  $1H$ : 1 $\beta$ ), 6.51 (d,  $J=10$  Hz,  $1H$ :  
 15  $NH$  2), from 6.80 to 6.90 (mt,  $3H$ :  $H$  2 -  $H$  4 and  $H$  6 of  
 the aromatic moiety in position 4), from 7.15 to 7.40  
 (mt,  $6H$ :  $H$  5 of the aromatic moiety in position 4 and  
 aromatic  $H$  6), 7.45 (broad d,  $J=9$  Hz,  $1H$ : 1' $H_4$ ), 7.50  
 (dd,  $J=9$  and  $4$  Hz,  $1H$ : 1' $H_5$ ), 7.80 (broad d,  $J = 4$  Hz,  
 20  $1H$ : 1' $H_6$ ), 8.40 (d,  $J=10$  Hz,  $1H$ :  $NH$  1), 8.73 (d,  
 $J=9.5$  Hz,  $1H$ :  $NH$  6), 11.62 (s,  $1H$ :  $OH$ ).

Using the fractions derived from the silica  
 column described above which contain the new derivative  
 of pristinamycin  $I_R$ , 7 mg of 4 $\epsilon$ -methoxy-de(4)-  
 25 dimethylamino)pristinamycin  $I_R$  (mass spectrometry:  $M+H^+$   
 $= 826$ ) are isolated by carrying out semi-preparative  
 column chromatography as described above.

**EXAMPLE 17: Preparation of 4ε-fluoro-4ζ-methyl-de(4ζ-dimethylamino)pristinamycin I<sub>A</sub>.**

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-3-fluoro-4-methylphenylalanine, synthesized as in Example 34-5, in 0.1N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I<sub>A</sub> are combined and evaporated. 15 mg of dry residue are obtained. This residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new

pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 9 mg of 4 $\epsilon$ -fluoro-4 $\zeta$ -methyl-de(4 $\zeta$ -

5 dimethylamino)pristinamycin I<sub>A</sub> are obtained.

NMR spectrum: <sup>1</sup>H (400 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm, ref. TMS): 0.60 (dd, J=16 and 5.5 Hz, 1H, 5  $\beta_2$ ), 0.91 (t, J=7.5 Hz, 3H: CH<sub>3</sub> 2  $\gamma$ ), 1.12 (mt, 1H: 3  $\beta_2$ ), from 1.25 to 1.35 (mt, 1H: 3  $\gamma_2$ ), 1.33 (d, J=7.5 Hz, 3H: CH<sub>3</sub> 1  $\gamma$ ), from 1.50 to 1.85 (mt, 3H: 3  $\gamma_1$  and CH<sub>2</sub> 2  $\beta$ ), 2.02 (mt, 1H, 3  $\beta_1$ ), 2.13 (mt, 1H, 5  $\delta_2$ ), 2.27 (s, 3H: ArCH<sub>3</sub>), 2.36 (broad d, J=16 Hz, 1H: 5  $\delta_1$ ), 2.45 (d, J=16 Hz, 1H: 5  $\beta_1$ ), 2.85 (dt, J=13 and 4.5 Hz, 1H: 5  $\epsilon_2$ ), 2.97 (dd, J=12.5 and 4.5 Hz, 1H: 4  $\beta_2$ ), 3.23 (s, 3H: NCH<sub>3</sub> 4), 3.30 and 3.56 (2 mts, 1H each: CH<sub>2</sub> 3  $\delta$ ), 3.37 (t, J=12.5 Hz, 1H: 4  $\beta_1$ ), 4.55 (t, J=7.5 Hz, 1H, 3  $\alpha$ ), 4.75 (broad dd, J=13 and 8 Hz, 1H: 5  $\epsilon_1$ ), 4.83 (mt, 1H: 2 $\alpha$ ), 4.89 (broad d, J=10 Hz, 1H: 1 $\alpha$ ), 5.29 (dd, J=12.5 and 4.5 Hz, 1H: 4  $\alpha$ ), 5.32 (broad d, J=5.5 Hz, 1H: 5  $\alpha$ ), 5.89 (d J=9.5 Hz, 1H: 6  $\alpha$ ), 5.92 (mt, 1H: 1 $\beta$ ), 6.49 (d, J=10 Hz, 1H: NH 2), 6.90 (mt, 2H: H 2 and H 6 of the aromatic moiety in position 4), 7.11 (t, J=8 Hz, 1H: H 5 of the aromatic moiety in position 4), from 7.10 to 7.30 (mt, 5H: aromatic H 6), 7.43 (dd, J=8.5 and 1 Hz, 1H: 1'H<sub>4</sub>), 7.49 (dd, J=8.5 and 4.5 Hz, 1H: 1'H<sub>5</sub>), 7.75 (dd, J=4.5 and 1 Hz, 1H: 1'H<sub>6</sub>), 8.48 (d, J=10 Hz, 1H: NH 1), 8.70 (d, J=9.5 Hz, 1H: NH 6), 11.60 (s, 1H: OH).



**EXAMPLE 18: Preparation of 4 $\zeta$ -ethylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub>**

Strain SP92::pVRC508 is cultured in production medium using 50 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-ethylaminophenylalanine dihydrochloride, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.5 litres of must recovered from the 50 erlenmeyer flasks are extracted with 2 volumes of a mixture of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica column (30 g) which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4 $\zeta$ -ethylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 65% water and 35% acetonitrile and injected onto a semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% acetonitrile. The fractions containing 4 $\zeta$ -ethylamino-de(4 $\zeta$ -

dimethylamino)pristinamycin I<sub>a</sub> are combined and  
 extracted with one volume of dichloromethane. The  
 organic phase is washed with water, dried over sodium  
 sulphate and then evaporated. 10 mg of 4 $\zeta$ -ethylamino-  
 5 de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>a</sub> are obtained.

NMR spectrum. <sup>1</sup>H (400 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm,  
 ref. TMS): 0.72 (dd, J = 16 and 6 Hz, 1H: 1H of the CH<sub>2</sub>  
 in 5  $\beta$ ); 0.90 (t, J = 7.5 Hz, 3H: CH<sub>3</sub> in 2  $\gamma$ ); 1.15 (mt,  
 1H: 1H of the CH<sub>2</sub> in 3  $\beta$ ); from 1.20 to 1.40 (mt, 1H: 1H  
 10 of the CH<sub>2</sub> in 3  $\gamma$ ); 1.27 (t, J = 7.5 Hz, 3H: CH<sub>3</sub> of the  
 ethyl); 1.33 (d, J = 7 Hz, 3H: CH<sub>3</sub> in 1  $\gamma$ ); from 1.50 to  
 1.65 (mt, 1H: the other H of the CH<sub>2</sub> in 3  $\gamma$ ); 1.60 and  
 1.74 (2 mts, 1H each: CH<sub>2</sub> in 2  $\beta$ ); 2.02 (mt, 1H: the  
 other H of CH<sub>2</sub> in 3  $\beta$ ); 2.21 and 2.33 (respectively, mt  
 15 and broad d, J = 16.5 Hz, 1H each: CH<sub>2</sub> in 5  $\delta$ ); 2.40 (d,  
 J = 16 Hz, 1H: the other H of the CH<sub>2</sub> in 5  $\beta$ ); 2.82 (dt,  
 J = 13 and 4.5 Hz, 1H: 1H of the CH<sub>2</sub> in 5  $\epsilon$ ); 2.89 (dd,  
 J = 12 and 4 Hz, 1H: 1H of the CH<sub>2</sub> in 4  $\beta$ ); 3.10 (mt,  
 2H: NCH<sub>2</sub> of the ethyl); from 3.20 to 3.35 (mt, 1H: 1H of  
 20 the CH<sub>2</sub> in 3  $\delta$ ); 3.26 (s, 3H: NCH<sub>3</sub>); 3.31 (t, J = 12 Hz,  
 1H: the other H of the CH<sub>2</sub> in 4  $\beta$ ); 3.54 (mt, 1H: the  
 other H of the CH<sub>2</sub> in 3  $\delta$ ); 3.67 (unres. comp., 1H: NH);  
 4.56 (dd, J = 6.5 and 7 Hz, 1H: 3  $\alpha$ ); 4.75 (broad dd, J  
 = 13 and 8 Hz, 1H: the other H of the CH<sub>2</sub> in 5  $\epsilon$ ); 4.84  
 25 (mt, 1H: 2  $\alpha$ ); 4.90 (broad d, J = 10 Hz, 1H : 1  $\alpha$ ) ;  
 5.24 (dd, J = 12 and 4 Hz, 1H: 4  $\alpha$ ); 5.32 (broad d, J =  
 6 Hz, 1H: 5  $\alpha$ ); 5.88 (d, J = 9.5 Hz, 1H : 6  $\alpha$ ); 5.90  
 (mt, 1H : 1  $\beta$ ); 6.52 (d, J = 8 Hz, 3H : NH in 2 and

aromatic H in 4  $\epsilon$ ); 7.00 (d,  $J = 8$  Hz, 2H : aromatic H in 4  $\delta$ ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.46 (limiting AB, 2H: 1'H<sub>4</sub> and 1'H<sub>5</sub>); 7.84 (dd,  $J = 4$  and 1 Hz, 1H: 1'H<sub>6</sub>); 8.45 (d,  $J = 10$  Hz, 1H: NH in 1);  
 5 8.77 (d,  $J = 9.5$  Hz, 1H: NH in 6); 11.65 (s, 1H: OH).

**EXAMPLE 19: Preparation of 4 $\zeta$ -diethylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub>**

Strain SP92::pVRC508 is cultured in production medium using 50 erlenmeyer flasks, as  
 10 described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-diethylaminophenylalanine dihydrochloride, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.5 litres of must recovered from the 50  
 15 erlenmeyer flasks are extracted with 2 volumes of a mixture of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and  
 20 then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica column (30 g) which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in  
 25 dichloromethane. The fractions containing 4 $\zeta$ -diethylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> are combined and evaporated. The dry residue is taken up in

7 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in two portions onto a semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of  
 5 68% 100 mM phosphate buffer, pH 2.9, and 32% acetonitrile. The fractions containing 4 $\zeta$ -diethylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I $_A$  are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium  
 10 sulphate and then evaporated. 50 mg of 4 $\zeta$ -diethylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I $_A$  are obtained.

NMR spectrum.  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm, ref. TMS): 0.65 (dd,  $J = 16$  and  $6$  Hz, 1H: 1H of the  $\text{CH}_2$  in 5  $\beta$ ); 0.90 (t,  $J = 7.5$  Hz, 3H:  $\text{CH}_3$  in 2  $\gamma$ ); 1.14 (t,  $J = 7$  Hz, 6H:  $\text{CH}_3$  of the ethyl); 1.15 (mt, 1H: 1H of the  $\text{CH}_2$  in 3  $\beta$ ); 1.26 (mt, 1H: 1H of the  $\text{CH}_2$  in 3  $\gamma$ ); 1.32 (d,  $J = 6.5$  Hz, 3H:  $\text{CH}_3$  in 1  $\gamma$ ); 1.55 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\gamma$ ); 1.63 and 1.75 (2 mts, 1H each:  $\text{CH}_2$  in 2  $\beta$ ); 2.02 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\beta$ );  
 15 2.22 and 2.31 (respectively, mt and broad d,  $J = 16.5$  Hz, 1H each:  $\text{CH}_2$  in 5  $\delta$ ); 2.37 (d,  $J = 16$  Hz, 1H: the other H of the  $\text{CH}_2$  in 5  $\beta$ ); 2.80 (dt,  $J = 13$  and  $4.5$  Hz, 1H: 1H of the  $\text{CH}_2$  in 5  $\epsilon$ ); 2.89 (dd,  $J = 12.5$  and  $4$  Hz, 1H: 1H of the  $\text{CH}_2$  in 4  $\beta$ ); from 3.20 to 3.40 (mt, 6H:  $\text{NCH}_2$  of the ethyl - 1H of the  $\text{CH}_2$  in 3  $\delta$  and the other H of the  $\text{CH}_2$  in 4  $\beta$ ); 3.27 (s, 3H:  $\text{NCH}_3$ ); 3.55 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\delta$ ); 4.58 (dd,  $J = 8$  and  $6$  Hz, 1H: 3  $\alpha$ ); 4.76 (broad dd,  $J = 13$  and  $7.5$  Hz, 1H: the  
 25

other H of the CH<sub>2</sub> in 5 ε); 4.84 (mt, 1H: 2 α); 4.89  
 (dd, J = 10 and 1 Hz, 1H: 1 α) ; 5.21 (dd, J = 12.5 and  
 4 Hz, 1H: 4 α); 5.28 (broad d, J = 6 Hz, 1H : 5 α);  
 5.87 (d, J = 9.5 Hz; 1H: 6 α); 5.90 (mt, 1H: 1 β); 6.52  
 5 (d, J = 9.5 Hz, 1H : NH in 2); 6.60 (d, J = 8 Hz, 2H:  
 aromatic H in 4 ε); 7.02 (d, J = 8 Hz, 2H: aromatic H  
 in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6);  
 7.46 (limiting AB, 2H: 1'H<sub>4</sub> and 1'H<sub>5</sub>); 7.88 (dd, J = 4.5  
 and 2.5 Hz, 1H: 1'H<sub>6</sub>); 8.43 (d, J = 10 Hz, 1H: NH in 1);  
 10 8.76 (d, J = 9.5 Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

**EXAMPLE 20: Preparation of 4}-diallylamino-  
 de(4}-dimethylamino)pristinamycin I<sub>A</sub>**

Strain SP92::pVRC508 is cultured in  
 production medium using 94 erlenmeyer flasks, as  
 15 described in Example 3, with 1 ml of a 20 g/l solution  
 of (R,S)-4-diallylaminophenylalanine dihydrochloride,  
 synthesized as in Example 38-1, in water being added at  
 16h. At the end of 40h of culture, the 2.8 litres of  
 must recovered from the 94 erlenmeyer flasks are  
 20 extracted with 2 volumes of a mixture consisting of 66%  
 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile,  
 and then centrifuged. The supernatant is extracted with  
 2 times 0.5 volumes of dichloromethane. The  
 chloromethylene phases are washed with water and then  
 25 combined, dried over sodium sulphate and evaporated.  
 The dry extract is taken up in 20 ml of dichloromethane  
 and injected onto a silica (30 g) column which is

mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4 $\zeta$ -diallylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I $_A$  are combined and

5 evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 $\mu$  C8 10 $\times$ 250 mm (Machery Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH

10 2.9, and 48% acetonitrile. The fractions containing 4 $\zeta$ -diallylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I $_A$  are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated.

15 15 mg of 4 $\zeta$ -diallylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I $_A$  are obtained.

NMR spectrum.  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm, ref.TMS): 0.55 (dd,  $J = 16$  and  $6$  Hz,  $1\text{H} : 1\text{H}$  of the  $\text{CH}_2$  in  $5 \beta$ ); 0.93 (t,  $J = 7.5$  Hz,  $3\text{H} : \text{CH}_3$  in  $2 \gamma$ ); 1.18 (mt,  $1\text{H} : 1\text{H}$  of the  $\text{CH}_2$  in  $3 \beta$ ); 1.25 (mt,  $1\text{H} : 1\text{H}$  of the  $\text{CH}_2$  in  $3 \gamma$ ); 1.34 (d,  $J = 6.5$  Hz,  $3\text{H} : \text{CH}_3$  in  $1 \gamma$ ); 1.59 (mt,  $1\text{H} : \text{the other H of the } \text{CH}_2 \text{ in } 3 \gamma$ ); 1.68 and 1.78 (2 mts,  $1\text{H}$  each:  $\text{CH}_2$  in  $2 \beta$ ); 2.04 (mt,  $1\text{H} : \text{the other H of } \text{CH}_2 \text{ in } 3 \beta$ ); 2.25 and 2.34 (respectively, mt and broad

20 d,  $J = 16.5$  Hz,  $1\text{H}$  each:  $\text{CH}_2$  in  $5 \delta$ ); 2.40 (d,  $J = 16$  Hz,  $1\text{H} : \text{the other H of the } \text{CH}_2 \text{ in } 5 \beta$ ); 2.83 (dt,  $J = 13$  and  $4.5$  Hz,  $1\text{H} : 1\text{H}$  of the  $\text{CH}_2$  in  $5 \epsilon$ ); 2.92 (dd,  $J = 12$  and  $4$  Hz,  $1\text{H} : 1\text{H}$  of the  $\text{CH}_2$  in  $4 \beta$ ); from 3.20 to 3.30

(mt, 1H: 1H of the CH<sub>2</sub> in 3 δ); 3.29 (s, 3H: NCH<sub>3</sub>); 3.33 (t, J = 12 Hz, 1H: the other H of the CH<sub>2</sub> in 4 β); 3.57 (mt, 1H: the other H of the CH<sub>2</sub> in 3 δ); 3.93 (limiting AB, 4H: NCH<sub>2</sub> of the allyl); 4.60 (dd, J = 8 and 6.5 Hz, 1H: 3 α); 4.78 (broad dd, J = 13 and 7.5 Hz, 1H: the other H of the CH<sub>2</sub> in 5 ε); 4.87 (mt, 1H: 2 α); 4.92 (dd, J = 10 and 1 Hz, 1H: 1 α); from 5.10 to 5.25 (mt, 5H: 4 α and =CH<sub>2</sub> of the allyl); 5.28 (broad d, J = 6 Hz, 1H: 5 α); 5.85 (mt, 2H: CH= of the allyl); 5.92 (d, J = 9.5 Hz, 1H: 6 α); 5.94 (mt, 1H : 1 β); 6.54 (d, J = 10 Hz, 1H: NH in 2); 6.65 (d, J = 8 Hz, 2H : aromatic H in 4 ε); 7.05 (d, J = 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.51 (limiting AB, 2H: 1'H<sub>4</sub> and 1'H<sub>5</sub>); 7.88 (dd, J = 4 and 2 Hz, 1H: 1'H<sub>6</sub>); 8.43 (d, J = 10 Hz, 1H: NH in 1); 8.77 (d, J = 9.5 Hz, 1H: NH in 6); 11.65 (s, 1H : OH).

**EXAMPLE 21: Preparation of 4}-allylethyl-amino-de(4}-dimethylamino)pristinamycin I<sub>A</sub>**

Strain SP92::pVRC508 is cultured in production medium using 26 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-allylethylaminophenylalanine dihydrochloride, synthesized as in Example 39-4, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 0.78 litre of must recovered from the 26 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM

phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4 $\zeta$ -allylethylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I $_A$  are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 $\mu$  C8 10 $\times$ 250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4 $\zeta$ -allylethylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I $_A$  are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 20 mg of 4 $\zeta$ -allylethylamino-de(4 $\zeta$ -dimethylamino)-pristinamycin I $_A$  are obtained.

NMR spectrum.  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm, ref. TMS): 0.58 (dd,  $J = 16$  and  $6$  Hz, 1H: 1H of  $\text{CH}_2$  in 5  $\beta$ ); 0.91 (t,  $J = 7.5$  Hz, 3H:  $\text{CH}_3$  in 2  $\gamma$ ); 1.16 (t,  $J = 7$  Hz, 3H:  $\text{CH}_3$  of the ethyl); 1.16 (mt, 1H: 1H of the  $\text{CH}_2$  in 3  $\beta$ ); 1.25 (mt, 1H: 1H of  $\text{CH}_2$  in 3  $\gamma$ ); 1.32



(d,  $J = 6.5$  Hz, 3H:  $\text{CH}_3$  in 1  $\gamma$ ); 1.54 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\gamma$ ); 1.63 and 1.75 (2 mts, 1H each:  $\text{CH}_2$  in 2  $\beta$ ); 2.02 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\beta$ ); 2.23 and 2.31 (respectively, mt and broad d,  $J = 16.5$  Hz, 1H each:  $\text{CH}_2$  in 5  $\delta$ ); 2.37 (d,  $J = 16$  Hz, 1H: the other H of the  $\text{CH}_2$  in 5  $\beta$ ); 2.80 (dt,  $J = 13$  and 4.5 Hz, 1H : 1H of  $\text{CH}_2$  in 5  $\epsilon$ ); 2.87 (dd,  $J = 12$  and 4 Hz, 1H: 1H of the  $\text{CH}_2$  in 4  $\beta$ ); from 3.15 to 3.30 (mt, 1H: 1H of the  $\text{CH}_2$  in 3  $\delta$ ); 3.26 (s, 3H:  $\text{NCH}_3$ ); 3.30 (t,  $J = 12$  Hz, 1H: the other H of  $\text{CH}_2$  in 4  $\beta$ ); 3.36 (mt, 2H:  $\text{NCH}_2$  of the ethyl); 3.54 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\delta$ ); 3.90 (limiting AB, 2H:  $\text{NCH}_2$  of the allyl); 4.57 (dd,  $J = 8$  and 6 Hz, 1H: 3  $\alpha$ ); 4.76 (broad dd,  $J = 13$  and 7.5 Hz, 1H: the other H of the  $\text{CH}_2$  in 5  $\epsilon$ ); 4.84 (mt, 1H: 2  $\alpha$ ); 4.89 (dd,  $J = 10$  and 1 Hz, 1H: 1  $\alpha$ ); from 5.05 to 5.20 (mt, 3H: 4  $\alpha$  and  $=\text{CH}_2$  of the allyl); 5.27 (broad d,  $J = 6$  Hz, 1H : 5  $\alpha$ ); 5.83 (mt, 1H:  $\text{CH}=\text{}$  of the allyl); 5.88 (d,  $J = 9.5$  Hz, 1H: 6  $\alpha$ ); 5.91 (mt, 1H: 1  $\beta$ ); 6.50 (d,  $J = 10$  Hz, 1H: NH in 2); 6.60 (d,  $J = 8$  Hz, 2H: aromatic H in 4  $\epsilon$ ); 7.02 (d,  $J = 8$  Hz, 2H: aromatic H in 4  $\delta$ ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.47 (limiting AB, 2H:  $1'\text{H}_4$  and  $1'\text{H}_5$ ); 7.88 (dd,  $J = 4$  and 2 Hz, 1H:  $1'\text{H}_6$ ); 8.41 (d,  $J = 10$  Hz, 1H: NH in 1); 8.75 (d,  $J = 9.5$  Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

**EXAMPLE 22: Preparation of the 4 $\beta$ -ethyl-propylamino-de(4 $\beta$ -dimethylamino)pristinamycin I<sub>A</sub>**

Strain SP92::pVRC508 is cultured in production medium using 60 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-ethylpropylaminophenylalanine dihydrochloride, synthesized as in Example 39-6, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.8 litre of must recovered from the 60 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4 $\beta$ -ethylpropylamino-de(4 $\beta$ -dimethylamino)pristinamycin I<sub>A</sub> are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 63% 100 mM phosphate buffer, pH 2.9, and 37% of acetonitrile. The fractions containing

4 $\zeta$ -ethylpropylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated.

5 16 mg of 4 $\zeta$ -ethylpropylamino-de(4 $\zeta$ -dimethylamino)-pristinamycin I<sub>A</sub> are obtained.

NMR spectrum. <sup>1</sup>H (400 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm, ref. TMS): 0.67 (dd, J = 16 and 6 Hz, 1H: 1H of the CH<sub>2</sub> in 5  $\beta$ ); 0.91 (t, J = 7.5 Hz, 3H: CH<sub>3</sub> in 2  $\gamma$ ); 0.95 (t, J = 7.5 Hz, 3H: CH<sub>3</sub> of propyl); 1.14 (t, J = 7 Hz, 3H: CH<sub>3</sub> of the ethyl); 1.15 (mt, 1H: 1H of the CH<sub>2</sub> in 3  $\beta$ ); 1.25 (mt, 1H: 1H of the CH<sub>2</sub> in 3  $\gamma$ ); 1.33 (d, J = 7 Hz, 3H: CH<sub>3</sub> in 1  $\gamma$ ); from 1.45 to 1.65 (mt, 3H: the other H of the CH<sub>2</sub> in 3  $\gamma$  and CH<sub>2</sub> propyl); 1.63 and 1.75 (2 mts, 1H each: CH<sub>2</sub> in 2  $\beta$ ); 2.02 (mt, 1H: the other H of the CH<sub>2</sub> in 3  $\beta$ ); 2.23 and 2.33 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH<sub>2</sub> in 5  $\delta$ ); 2.37 (d, J = 16 Hz, 1H: the other H of the CH<sub>2</sub> in 5  $\beta$ ); 2.80 (dt, J = 13 and 5 Hz, 1H: 1H of the CH<sub>2</sub> in 5  $\epsilon$ ); 2.89 (dd, J = 12 and 4 Hz, 1H: 1H of the CH<sub>2</sub> in 4  $\beta$ ); from 3.10 to 3.25 (mt, 3H: 1H of the CH<sub>2</sub> in 3  $\delta$  and NCH<sub>2</sub> of the propyl); 3.26 (s, 3H: NCH<sub>3</sub>); from 3.25 to 3.40 (mt, 2H: NCH<sub>2</sub> of the ethyl); 3.34 (t, J = 12 Hz, 1H: the other H of the CH<sub>2</sub> in 4  $\beta$ ); 3.54 (mt, 1H: the other H of the CH<sub>2</sub> in 3  $\delta$ ); 4.57 (dd, J = 7.5 and 6 Hz, 1H: 3  $\alpha$ ); 4.76 (broad dd, J = 13 and 7.5 Hz, 1H: the other H of the CH<sub>2</sub> in 5  $\epsilon$ ); 4.84 (mt, 1H: 2  $\alpha$ ); 4.89 (dd, J = 10 and 1 Hz, 1H: 1  $\alpha$ ); 5.21 (dd, J = 12 and 4 Hz, 1H: 4  $\alpha$ ); 5.28 (broad

d, J = 6 Hz, 1H: 5  $\alpha$ ); 5.88 (d, J = 9.5 Hz, 1H: 6  $\alpha$ );  
 5.91 (mt, 1H: 1  $\beta$ ); 6.48 (d, J = 10 Hz, 1H: NH in 2);  
 6.60 (d, J = 8 Hz, 2H: aromatic H in 4  $\epsilon$ ); 7.03 (d, J =  
 8 Hz, 2H: aromatic H in 4  $\delta$ ); from 7.10 to 7.35 (mt,  
 5 5H: aromatic H in 6); 7.47 (limiting AB, 2H: 1'H<sub>4</sub> and  
 1'H<sub>5</sub>); 7.89 (mt, 1H: 1'H<sub>6</sub>); 8.42 (d, J = 10 Hz, 1H : NH  
 in 1); 8.76 (d, J = 9.5 Hz, 1H: NH in 6); 11.62 (s, 1H:  
 OH).

**EXAMPLE 23: Preparation of the 4 $\zeta$ -trifluoro-**  
 10 **methoxy-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub>**  
 Strain SP92::pVRC508 is cultured in  
 production medium using 60 erlenmeyer flasks, as  
 described in Example 3, with 1 ml of a 20 g/l solution  
 of (R,S)-4-O-trifluoromethyltyrosine hydrochloride,  
 15 synthesized as in Example 34-8, in water being added at  
 16h. At the end of 40h of culture, the 1.8 litres of  
 must recovered from the 60 erlenmeyer flasks is  
 extracted with 2 volumes of a mixture consisting of 66%  
 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile,  
 20 and then centrifuged. The supernatant is extracted with  
 2 times [lacuna] volumes of dichloromethane. The  
 chloromethylene phases are washed with water and then  
 combined, dried over sodium sulphate and evaporated.  
 The dry extract is taken up in [lacuna] ml of  
 25 dichloromethane and injected onto a silica (30 g)  
 column which is mounted in dichloromethane and eluted  
 successively with plateaus of from 0 to 10% methanol in

dichloromethane. The fractions containing 4 $\zeta$ -trifluoromethoxy-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected in two portions onto a semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% of acetonitrile. The fractions containing 4 $\zeta$ -trifluoromethoxy-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 46.5 mg of 4 $\zeta$ -trifluoromethoxy-de(4 $\zeta$ -dimethylamino)-pristinamycin I<sub>A</sub> are obtained.

NMR spectrum. <sup>1</sup>H (400 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm, ref. TMS): 0.77 (dd,  $J$  = 16 and 5.5 Hz, 1H: 1H of the CH<sub>2</sub> in 5  $\beta$ ); 0.92 (t,  $J$  = 7.5 Hz, 3H: CH<sub>3</sub> in 2  $\gamma$ ); 1.08 (mt, 1H: 1H of the CH<sub>2</sub> in 3  $\beta$ ); from 1.30 to 1.40 (mt, 1H: 1H of the CH<sub>2</sub> in 3  $\gamma$ ); 1.33 (d,  $J$  = 7 Hz, 3H: CH<sub>3</sub> in 1  $\gamma$ ); from 1.55 to 1.70 (mt, 1H: the other H of the CH<sub>2</sub> in 3  $\gamma$ ); 1.65 and 1.76 (2 mts, 1H each: CH<sub>2</sub> in 2  $\beta$ ); 2.02 (mt, 1H: the other H of the CH<sub>2</sub> in 3  $\beta$ ); 2.11 and 2.40 (respectively, mt and broad d,  $J$  = 16.5 Hz, 1H each: CH<sub>2</sub> in 5  $\delta$ ); 2.54 (d,  $J$  = 16 Hz, 1H: the other H of the CH<sub>2</sub> in 5  $\beta$ ); 2.88 (dt,  $J$  = 13 and 4 Hz, 1H: 1H of the CH<sub>2</sub> in 5  $\epsilon$ ); 3.08 (dd,  $J$  = 12 and 5 Hz, 1H: 1H of the CH<sub>2</sub> in 4  $\beta$ ); 3.22 (s, 3H: NCH<sub>3</sub>); from 3.30 to 3.45

(mt, 1H: 1H of the CH<sub>2</sub> in 3 δ); 3.39 (t, J = 12 Hz, 1H: the other H of the CH<sub>2</sub> in 4 β); 3.59 (mt, 1H: the other H of the CH<sub>2</sub> in 3 δ); 4.53 (t, J = 7.5 Hz, 1H : 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH<sub>2</sub> in 5 ε); 4.85 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1.5 Hz, 1H: 1 α); 5.35 (broad d, J = 5.5 Hz, 1H: 5 α); 5.41 (dd, J = 12 and 5 Hz, 1H: 4 α); 5.92 (d, J = 10 Hz, 1H : 6 α); 5.93 (mt, 1H: 1 β); 6.53 (d, J = 9.5 Hz, 1H: NH in 2); from 7.15 to 7.35 (mt, 5H: aromatic H in 6); 7.16 (d, J = 8 Hz, 2H: aromatic H in 4 ε); 7.26 (d, J = 8 Hz, 2H: aromatic H in 4 δ); 7.37 (dd, J = 8.5 and 4 Hz, 1H: 1'H<sub>5</sub>); 7.42 (dd, J = 8.5 and 1.5 Hz, 1H: 1'H<sub>4</sub>); 7.70 (dd, J = 4 and 1.5 Hz, 1H: 1'H<sub>6</sub>); 8.37 (d, J = 10 Hz, 1H: NH in 1); 8.75 (d, J = 10 Hz, 1H: NH in 6); 11.66 (s, 1H: OH).

**EXAMPLE 24: Preparation of 4'-allyloxy-de(4'-dimethylamino)pristinamycin I<sub>A</sub>**

Strain SP92::pVRC508 is cultured in production medium using 90 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (S)-4-O-allyltyrosine hydrochloride, synthesized as in Example 33, in 0.1N hydrochloric acid being added at 16h. At the end of 40h of culture, the 2.7 litres of must recovered from the 90 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with

2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4 $\zeta$ -allyloxy-de(4 $\zeta$ -dimethyl-amino)pristinamycin I $_A$  are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 $\mu$  C8 10 $\times$ 250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4 $\zeta$ -allyloxy-de(4 $\zeta$ -dimethylamino)pristinamycin I $_A$  are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 29 mg of 4 $\zeta$ -allyloxy-de(4 $\zeta$ -dimethylamino)pristinamycin I $_A$  are obtained.

NMR spectrum.  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm, ref. TMS): 0.63 (dd,  $J = 16$  and  $6$  Hz, 1H: 1H of  $\text{CH}_2$  in 5  $\beta$ ); 0.91 (t,  $J = 7.5$  Hz, 3H:  $\text{CH}_3$  in 2  $\gamma$ ); 1.13 (mt, 1H: 1H of  $\text{CH}_2$  in 3  $\beta$ ); 1.29 (mt, 1H: 1H of  $\text{CH}_2$  in 3  $\gamma$ ); 1.33 (d,  $J = 6.5$  Hz, 3H:  $\text{CH}_3$  in 1  $\gamma$ ); 1.57 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\gamma$ ); 1.65 and 1.74 (2 mts, 1H each:  $\text{CH}_2$  in 2  $\beta$ ); 2.02 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\beta$ );

2.14 and 2.34 (respectively, mt and broad d,  $J = 16.5$  Hz, 1H each:  $\text{CH}_2$  in 5  $\delta$ ); 2.43 (d,  $J = 16$  Hz, 1H: the other H of the  $\text{CH}_2$  in 5  $\beta$ ); 2.85 (dt,  $J = 13$  and 4 Hz, 1H: 1H of the  $\text{CH}_2$  in 5  $\epsilon$ ); 2.95 (dd,  $J = 12$  and 4 Hz, 1H: 1H of the  $\text{CH}_2$  in 4  $\beta$ ); 3.25 (s, 3H:  $\text{NCH}_3$ ); 3.33 (mt, 1H: 1H of the  $\text{CH}_2$  in 3  $\delta$ ); 3.36 (t,  $J = 12$  Hz, 1H: the other H of the  $\text{CH}_2$  in 4  $\beta$ ); 3.56 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\delta$ ); 4.51 (limiting AB, 2H:  $\text{OCH}_2$  of the allyl); 4.56 (t,  $J = 7.5$  Hz, 1H: 3  $\alpha$ ); 4.75 (broad dd,  $J = 13$  and 8 Hz, 1H: the other H of the  $\text{CH}_2$  in 5  $\epsilon$ ); 4.84 (mt, 1H: 2  $\alpha$ ); 4.88 (dd,  $J = 10$  and 1 Hz, 1H: 1  $\alpha$ ); 5.27 (dd,  $J = 12$  and 4 Hz, 1H: 4  $\alpha$ ); 5.32 (broad d,  $J = 6$  Hz, 1H: 5  $\alpha$ ); 5.30 and 5.40 (respectively, mt and dd,  $J = 17$  and 1.5 Hz, 1H each:  $=\text{CH}_2$  of the allyl); 5.89 (d,  $J = 9.5$  Hz, 1H: 6  $\alpha$ ); 5.91 (mt, 1H: 1  $\beta$ ); 6.02 (mt, 1H:  $\text{CH}=\text{}$  of the allyl); 6.50 (d,  $J = 10$  Hz, 1H: NH in 2); 6.85 (d,  $J = 8$  Hz, 2H: aromatic H in 4  $\epsilon$ ); 7.12 (d,  $J = 8$  Hz, 2H: aromatic H in 4  $\delta$ ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.45 (dd,  $J = 8.5$  and 1.5 Hz, 1H: 1' $\text{H}_4$ ); 7.57 (dd,  $J = 8.5$  and 4 Hz, 1H: 1' $\text{H}_5$ ); 7.77 (dd,  $J = 4$  and 1.5 Hz, 1H: 1' $\text{H}_6$ ); 8.41 (d,  $J = 10$  Hz, 1H: NH in 1); 8.74 (d,  $J = 9.5$  Hz, 1H: NH in 6); 11.63 (s, 1H: OH).

**EXAMPLE 25: Preparation of 4 $\zeta$ -ethoxy-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>1</sub>**

Strain SP92::pVRC508 is cultured in production medium using 90 erlenmeyer flasks, as



described in Example 3, with 1 ml of a 20 g/l solution of (S)-4-O-ethyltyrosine hydrochloride, synthesized as in Example 33, in 0.1N hydrochloric acid being added at 16h. At the end of 40h of culture, the 2.7 litres of must recovered from the 90 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4 $\zeta$ -ethoxy-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4 $\zeta$ -ethoxy-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 29 mg of 4 $\zeta$ -ethoxy-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> are obtained.

NMR spectrum.  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm, ref. TMS): 0.64 (dd,  $J = 16$  and  $5.5$  Hz, 1H: 1H of the  $\text{CH}_2$  in 5  $\beta$ ); 0.90 (t,  $J = 7.5$  Hz, 3H:  $\text{CH}_3$  in 2  $\gamma$ ); 1.12 (mt, 1H: 1H of the  $\text{CH}_2$  in 3  $\beta$ ); 1.25 (mt, 1H: 1H of the  $\text{CH}_2$  in 3  $\gamma$ ); 1.33 (d,  $J = 7$  Hz, 3H:  $\text{CH}_3$  in 1  $\gamma$ ); 1.42 (t,  $J = 7$  Hz, 3H:  $\text{CH}_3$  of the ethyl); 1.57 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\gamma$ ); 1.63 and 1.74 (2 mts, 1H each:  $\text{CH}_2$  in 2  $\beta$ ); 2.02 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\beta$ ); 2.16 and 2.35 (respectively mt and broad d,  $J = 16.5$  Hz, 1H each:  $\text{CH}_2$  in 5  $\delta$ ); 2.43 (d,  $J = 16$  Hz, 1H: the other H of the  $\text{CH}_2$  in 5  $\beta$ ); 2.83 (dt,  $J = 13$  and  $4$  Hz, 1H: 1H of the  $\text{CH}_2$  in 5  $\epsilon$ ); 2.93 (dd,  $J = 12$  and  $4$  Hz, 1H: 1H of the  $\text{CH}_2$  in 4  $\beta$ ); from 3.15 to 3.30 (mt, 1H: 1H of the  $\text{CH}_2$  in 3  $\delta$ ); 3.24 (s, 3H:  $\text{NCH}_3$ ); 3.35 (t,  $J = 12$  Hz, 1H: the other H of the  $\text{CH}_2$  in 4  $\beta$ ); 3.55 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\delta$ ); 3.95 (limiting AB, 2H:  $\text{OCH}_2$  of the ethyl); 4.56 (dd,  $J = 7.5$  and  $6$  Hz, 1H: 3  $\alpha$ ); 4.75 (broad dd,  $J = 13$  and  $8$  Hz, 1H: the other H of the  $\text{CH}_2$  in 5  $\epsilon$ ); 4.84 (mt, 1H: 2  $\alpha$ ); 4.87 (dd,  $J = 10$  and  $1$  Hz, 1H: 1  $\alpha$ ); 5.26 (dd,  $J = 12$  and  $4$  Hz, 1H: 4  $\alpha$ ); 5.32 (broad d,  $J = 5.5$  Hz, 1H: 5  $\alpha$ ); 5.88 (d,  $J = 10$  Hz, 1H: 6  $\alpha$ ); 5.92 (mt, 1H: 1  $\beta$ ); 6.48 (d,  $J = 10$  Hz, 1H: NH in 2); 6.83 (d,  $J = 8$  Hz, 2H: aromatic H in 4  $\epsilon$ ); 7.10 (d,  $J = 8$  Hz, 2H: aromatic H in 4  $\delta$ ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.44 (dd,  $J = 8.5$  and  $1.5$  Hz, 1H:  $1'\text{H}_4$ ); 7.57 (dd,  $J = 8.5$  and  $4.5$  Hz, 1H:  $1'\text{H}_5$ ); 7.77 (dd,  $J = 4.5$  and  $1.5$  Hz, 1H:  $1'\text{H}_6$ ); 8.38 (d,  $J = 10$  Hz, 1H: NH in 1); 8.75 (d,  $J = 10$  Hz, 1H :

NH in 6); 11.60 (s, 1H: OH).

**EXAMPLE 26: Preparation of 4 $\zeta$ -(2-chloroethoxy)-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub>**

Strain SP92::pVRC508 is cultured in  
5 production medium using 60 erlenmeyer flasks, as  
described in Example 3, with 1 ml of a 20 g/l solution  
of (S)-4-O-(2-chloroethyl)tyrosine hydrochloride,  
synthesized as in Example 42-1, in water being added at  
16h. At the end of 40h of culture, the 1.8 litres of  
10 must recovered from the 60 erlenmeyer flasks is  
extracted with 2 volumes of a mixture consisting of 66%  
100 mM phosphate buffer, pH 2.9, and 34% acetonitrile,  
and then centrifuged. The supernatant is extracted with  
2 times 0.5 volumes of dichloromethane. The  
15 chloromethylene phases are washed with water and then  
combined, dried over sodium sulphate and evaporated.  
The dry extract is taken up in 20 ml of dichloromethane  
and injected onto a silica (30 g) column which is  
mounted in dichloromethane and eluted successively with  
20 plateaus of from 0 to 10% methanol in dichloromethane.  
The fractions containing 4 $\zeta$ -(2-chloroethoxy)-de(4 $\zeta$ -  
dimethylamino)pristinamycin I<sub>A</sub> are combined and  
evaporated. The dry residue is taken up in 7 ml of a  
mixture consisting of 60% of water and 40% acetonitrile  
25 and injected onto a semi-preparative Nucleosil 7 $\mu$  C8  
10 $\times$ 250 mm (Macherey Nagel) column, which is eluted with  
a mixture consisting of 60% 100 mM phosphate buffer, pH

2.9, and 40% of acetonitrile. The fractions containing 4 $\beta$ -(2-chloroethoxy)-de(4 $\beta$ -dimethylamino)pristinamycin I $_A$  are combined and extracted with one volume of dichloromethane. The organic phase is washed with  
 5 water, dried over sodium sulphate and then evaporated. 3.2 mg of 4 $\beta$ -(2-chloroethoxy)-de(4 $\beta$ -dimethylamino)-pristinamycin I $_A$  are obtained.

NMR spectrum.  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm, ref. TMS): 0.66 (dd,  $J = 16$  and  $5.5$  Hz, 1H: 1H of the  
 10  $\text{CH}_2$  in 5  $\beta$ ); 0.91 (t,  $J = 7.5$  Hz, 3H:  $\text{CH}_3$  in 2  $\gamma$ ); 1.13 (mt, 1H: 1H of the  $\text{CH}_2$  in 3  $\beta$ ); 1.28 (mt, 1H: 1H of the  $\text{CH}_2$  in 3  $\gamma$ ); 1.33 (d,  $J = 7$  Hz, 3H:  $\text{CH}_3$  in 1  $\gamma$ ); 1.57 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\gamma$ ); 1.66 and 1.76 (2 mts, 1H each:  $\text{CH}_2$  in 2  $\beta$ ); 2.02 (mt, 1H: the other H  
 15 of the  $\text{CH}_2$  in 3  $\beta$ ); 2.16 and 2.37 (respectively, mt and broad d,  $J = 16.5$  Hz, 1H each:  $\text{CH}_2$  in 5  $\delta$ ); 2.47 (d,  $J = 16$  Hz, 1H: the other H of the  $\text{CH}_2$  in 5  $\beta$ ); 2.86 (dt,  $J = 13$  and  $4$  Hz, 1H: 1H of the  $\text{CH}_2$  in 5  $\epsilon$ ); 2.95 (dd,  $J = 12$  and  $4$  Hz, 1H: 1H of the  $\text{CH}_2$  in 4  $\beta$ ); 3.23 (s, 3H:  $\text{NCH}_3$ );  
 20 3.32 (mt, 1H: 1H of the  $\text{CH}_2$  in 3  $\delta$ ); 3.37 (t,  $J = 12$  Hz, 1H: the other H of the  $\text{CH}_2$  in 4  $\beta$ ); 3.57 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\delta$ ); 3.82 (t,  $J = 6$  Hz, 2H:  $\text{CH}_2\text{Cl}$ ); 4.19 (limiting AB, 2H:  $\text{OCH}_2$  of the ethyl); 4.55 (dd,  $J = 7.5$  and  $7$  Hz, 1H: 3  $\alpha$ ); 4.75 (broad dd,  $J = 13$   
 25 and  $8$  Hz, 1H: the other H of the  $\text{CH}_2$  in 5  $\epsilon$ ); 4.84 (mt, 1H: 2  $\alpha$ ); 4.87 (broad d,  $J = 10$  Hz, 1H: 1  $\alpha$ ); 5.28 (dd,  $J = 12$  and  $4$  Hz, 1H: 4  $\alpha$ ); 5.32 (broad d,  $J = 5.5$  Hz, 1H: 5  $\alpha$ ); 5.88 (d,  $J = 10$  Hz, 1H: 6 $\alpha$ ); 5.90 (mt, 1H:

1  $\beta$ ); 6.50 (d,  $J = 10$  Hz, 1H: NH in 2); 6.86 (d,  $J = 8$   
 Hz, 2H: aromatic H in 4  $\epsilon$ ); 7.13 (d,  $J = 8$  Hz, 2H:  
 aromatic H in 4  $\delta$ ); from 7.10 to 7.35 (mt, 5H: aromatic  
 H in 6); 7.45 (limiting AB, 2H: 1'H<sub>4</sub> and 1'H<sub>5</sub>); 7.75  
 5 (dd,  $J = 4$  and 2 Hz, 1H: 1'H<sub>6</sub>); 8.38 (d,  $J = 10$  Hz, 1H:  
 NH in 1); 8.74 (d,  $J = 10$  Hz, 1H: NH in 6); 11.62 (s,  
 1H: OH).

**EXAMPLE 27: Preparation of 4 $\beta$ -acetyl-de 4 $\beta$ -  
 dimethylamino)pristinamycin I<sub>a</sub>**

10 Strain SP92::pVRC508 is cultured in  
 production medium using 60 erlenmeyer flasks, as  
 described in Example 3, with 1 ml of a 20 g/l solution  
 of (S)-4-acetylphenylalanine, synthesized as in Example  
 33, in 0.1N sodium hydroxide solution being added at  
 15 16h. At the end of 40h of culture, the 1.8 litres of  
 must recovered from the 60 erlenmeyer flasks is  
 extracted with 2 volumes of a mixture consisting of 66%  
 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile,  
 and then centrifuged. The supernatant is extracted with  
 20 2 times 0.5 volumes of dichloromethane. The  
 chloromethylene phases are washed with water and then  
 combined, dried over sodium sulphate and evaporated.  
 The dry extract is taken up in 20 ml of dichloromethane  
 and injected onto a silica (30 g) column which is  
 25 mounted in dichloromethane and eluted successively with  
 plateaus of from 0 to 10% methanol in dichloromethane.  
 The fractions containing 4 $\beta$ -acetyl)-de(4 $\beta$ -dimethyl-

TOP SECRET 4752360

amino)pristinamycin I<sub>A</sub> are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm (Macherey  
 5 Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% of acetonitrile. The fractions containing 4 $\zeta$ -acetyl-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> are combined and extracted with one volume of dichloromethane. The  
 10 organic phase is washed with water, dried over sodium sulphate and then evaporated. 4.2 mg of 4 $\zeta$ -acetyl-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> are obtained.

NMR spectrum. <sup>1</sup>H (400 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm, ref. TMS): 0.73 (dd, J = 16 and 6 Hz, 1H: 1H of the CH<sub>2</sub>  
 15 in 5  $\beta$ ); 0.93 (t, J = 7.5 Hz, 3H: CH<sub>3</sub> in 2  $\gamma$ ); 1.12 (mt, 1H: 1H of the CH<sub>2</sub> in 3  $\beta$ ); from 1.25 to 1.45 (mt, 1H: 1H of the CH<sub>2</sub> in 3  $\gamma$ ); 1.33 (d, J = 7 Hz, 3H: CH<sub>3</sub> in 1  $\gamma$ ); 1.62 (mt, 1H: the other H of the CH<sub>2</sub> in 3  $\gamma$ ); from 1.60 to 1.85 (mt, 2H: CH<sub>2</sub> in 2  $\beta$ ); 2.02 (mt, 1H: the other H  
 20 of the CH<sub>2</sub> in 3  $\beta$ ); 2.20 and 2.42 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH<sub>2</sub> in 5  $\delta$ ); 2.52 (d, J = 16 Hz, 1H: the other H of the CH<sub>2</sub> in 5  $\beta$ ); 2.60 (s, 3H: ArCOCH<sub>3</sub>); 2.88 (dt, J = 13 and 4.5 Hz, 1H: 1H of CH<sub>2</sub> in 5  $\epsilon$ ); 3.13 (dd, J = 13.5 and 5.5 Hz, 1H: 1H of the CH<sub>2</sub>  
 25 in 4  $\beta$ ); 3.21 (s, 3H: NCH<sub>3</sub>); from 3.30 to 3.50 (mt, 1H: the other H of the CH<sub>2</sub> in 4  $\beta$ ); from 3.30 to 3.50 and 3.63 (2 mts, 1H each: CH<sub>2</sub> in 3  $\delta$ ); 4.53 (t, J = 7.5 Hz, 1H: 3  $\alpha$ ); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the

FOUO-4792360

other H of the CH<sub>2</sub> in 5  $\epsilon$ ); 4.84 (mt, 1H: 2  $\alpha$ ); 4.88 (dd, J = 10 and 1 Hz, 1H: 1  $\alpha$ ); 5.35 (broad d, J = 6 Hz, 1H: 5  $\alpha$ ); 5.43 (dd, J = 10.5 and 4 Hz, 1H: 4  $\alpha$ ); 5.90 (d, J = 9.5 Hz, 1H: 6  $\alpha$ ); 5.92 (mt, 1H: 1  $\beta$ ); 6.56 (d, J = 9.5 Hz, 1H: NH in 2); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.28 (d, J = 8 Hz, 2H: aromatic H in 4  $\delta$ ); 7.38 (dd, J = 8.5 and 2 Hz, 1H: 1'H<sub>4</sub>); 7.42 (dd, J = 8.5 and 4.5 Hz, 1H: 1'H<sub>5</sub>); 7.66 (dd, J = 4.5 and 2 Hz, 1H: 1'H<sub>6</sub>); 7.88 (d, J = 8 Hz, 2H: aromatic H in 4  $\epsilon$ ); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.74 (d, J = 9.5 Hz, 1H: NH in 6); 11.65 (s, 1H: OH).

**EXAMPLE 28: Preparation of 4 $\epsilon$ -dimethylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>1</sub>.**

Strain SP92::pVRC508 is cultured in production medium using 60 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-3-dimethylaminophenylalanine dihydrochloride, synthesized as in Example 35-10, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.8 litres of must recovered from the 60 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in

20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4 $\epsilon$ -dimethylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> are combined and evaporated. The dry residue is taken up in 3 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 $\mu$  C8 10 $\times$ 250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 57% 100 mM phosphate buffer, pH 2.9, and 43% of acetonitrile. The fractions containing 4 $\epsilon$ -dimethylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 1.1 mg of 4 $\epsilon$ -dimethylamino-de(4 $\zeta$ -dimethylamino)pristinamycin I<sub>A</sub> are obtained.

NMR spectrum. <sup>1</sup>H (400 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm, ref. TMS): 0.63 (dd, J = 16 and 5 Hz, 1H: 1H of the CH<sub>2</sub> in 5  $\beta$ ); 0.91 (t, J = 7.5 Hz, 3H: CH<sub>3</sub> in 2  $\gamma$ ); 1.13 (mt, 1H: 1H of the CH<sub>2</sub> in 3  $\beta$ ); from 1.20 to 1.35 (mt, 1H: 1H of the CH<sub>2</sub> in 3  $\gamma$ ); 1.32 (d, J = 6.5 Hz, 3H: CH<sub>3</sub> in 1  $\gamma$ ); 1.57 (mt, 1H: the other H of the CH<sub>2</sub> in 3  $\gamma$ ); 1.63 and 1.76 (2 mts, 1H each: CH<sub>2</sub> in 2  $\beta$ ); 2.02 (mt, 1H: the other H of the CH<sub>2</sub> in 3  $\beta$ ); 2.08 and 2.31 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH<sub>2</sub> in 5  $\delta$ ); 2.35 (d, J = 16 Hz, 1H: the other H of the CH<sub>2</sub> in 5  $\beta$ ); 2.81 (dt, J = 13 and 4 Hz, 1H: 1H of the CH<sub>2</sub> in 5  $\epsilon$ ); 2.90



(s, 6H:  $N(CH_3)_2$ ); 2.97 (dd,  $J = 12$  and 4 Hz, 1H: 1H of the  $CH_2$  in 4  $\beta$ ); from 3.20 to 3.30 (mt, 1H: 1H of the  $CH_2$  in 3  $\delta$ ); 3.28 (s, 3H:  $NCH_3$ ); 3.37 (t,  $J = 12$  Hz, 1H: the other H of the  $CH_2$  in 4  $\beta$ ); 3.57 (mt, 1H: the other H of the  $CH_2$  in 3  $\delta$ ); 4.58 (t,  $J = 7.5$  Hz, 1H : 3  $\alpha$ ); 4.74 (broad dd,  $J = 13$  and 8 Hz, 1H: the other H of the  $CH_2$  in 5  $\epsilon$ ); 4.86 (mt, 1H: 2  $\alpha$ ); 4.89 (broad d,  $J = 10$  Hz, 1H: 1  $\alpha$ ); 5.27 (dd,  $J = 12$  and 4 Hz, 1H: 4  $\alpha$ ); 5.29 (broad d,  $J = 5$  Hz, 1H : 5  $\alpha$ ); 5.89 (d,  $J = 9.5$  Hz, 1H: 6  $\alpha$ ); 5.90 (mt, 1H: 1  $\beta$ ); 6.50 (d,  $J = 10$  Hz, 1H: NH in 2); from 6.50 to 6.70 (mt, 3H: aromatic Hs in the ortho and in the para positions with respect to the dimethylamino); from 7.15 to 7.35 (mt, 5H: aromatic Hs in 6); 7.20 (t,  $J =$  [lacuna]

105111-413660

ml of a 20 g/l solution of (R,S)-3-methylthiophenyl-  
alanine hydrochloride, synthesized as in Example 34-11,  
in 0.1N sodium hydroxide solution being added at 16h.  
At the end of 40h of culture, the 1.68 litres of must  
5 recovered from the 56 erlenmeyer flasks is extracted  
with 2 volumes of a mixture consisting of 66% 100 mM  
phosphate buffer, pH 2.9, and 34% acetonitrile, and  
then centrifuged. The supernatant is extracted with 2  
times 0.5 volumes of dichloromethane. The  
10 chloromethylene phases are washed with water and then  
combined, dried over sodium sulphate and evaporated.  
The dry extract is taken up in 20 ml of dichloromethane  
and injected onto a silica (30 g) column which is  
mounted in dichloromethane and eluted successively with  
15 plateaus of from 0 to 10% methanol in dichloromethane.  
The fractions containing the novel derivative of  
pristinamycin I<sub>a</sub> are combined and evaporated. The dry  
residue is taken up in 7 ml of a mixture consisting of  
54% of water and 46% acetonitrile and injected onto a  
20 semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm (Macherey  
Nagel) column, which is eluted with a mixture  
consisting of 55% 100 mM phosphate buffer, pH 2.9, and  
45% of acetonitrile. The fractions containing the novel  
pristinamycin are combined and extracted with one  
25 volume of dichloromethane. The organic phase is washed  
with water, dried over sodium sulphate and then  
evaporated. 20 mg of 4 $\epsilon$ -methylthio-de(4 $\gamma$ -dimethyl-  
amino)pristinamycin I<sub>a</sub> are obtained.

NMR spectrum.  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm, ref. TMS): 0.56 (dd,  $J = 16$  and  $5.5$  Hz,  $1\text{H}$ :  $1\text{H}$  of the  $\text{CH}_2$  in  $5\ \beta$ ); 0.90 (t,  $J = 7.5$  Hz,  $3\text{H}$ :  $\text{CH}_3$  in  $2\ \gamma$ ); 1.13 (mt,  $1\text{H}$ :  $1\text{H}$  of the  $\text{CH}_2$  in  $3\ \beta$ ); 1.28 (mt,  $1\text{H}$ :  $1\text{H}$  of the  $\text{CH}_2$  in  $3\ \gamma$ ); 1.32 (d,  $J = 6.5$  Hz,  $3\text{H}$ :  $\text{CH}_3$  in  $1\ \gamma$ ); 1.58 (mt,  $1\text{H}$ : the other H of the  $\text{CH}_2$  in  $3\ \gamma$ ); 1.62 and 1.74 (2 mts,  $1\text{H}$  each:  $\text{CH}_2$  in  $2\ \beta$ ); 2.02 (mt,  $1\text{H}$ : the other H of the  $\text{CH}_2$  in  $3\ \beta$ ); 2.25 and 2.35 (respectively, mt and broad d,  $J = 16.5$  Hz,  $1\text{H}$  each:  $\text{CH}_2$  in  $5\ \delta$ ); 2.39 (d,  $J = 16$  Hz,  $1\text{H}$ : the other H of the  $\text{CH}_2$  in  $5\ \beta$ ); 2.43 (s,  $3\text{H}$ :  $\text{SCH}_3$ ); 2.82 (dt,  $J = 13$  and  $4$  Hz,  $1\text{H}$ :  $1\text{H}$  of the  $\text{CH}_2$  in  $5\ \epsilon$ ); 2.98 (dd,  $J = 12$  and  $4.5$  Hz,  $1\text{H}$ :  $1\text{H}$  of the  $\text{CH}_2$  in  $4\ \beta$ ); 3.26 (s,  $3\text{H}$ :  $\text{NCH}_3$ ); 3.30 (t,  $J = 12$  Hz  $1\text{H}$ :  $1\text{H}$  of  $\text{CH}_2$  in  $3\ \delta$ ); 3.38 (mt,  $1\text{H}$ : the other H of the  $\text{CH}_2$  in  $4\ \beta$ ); 3.57 (mt,  $1\text{H}$ : the other H of the  $\text{CH}_2$  in  $3\ \delta$ ); 4.56 (t,  $J = 7.5$  Hz,  $1\text{H}$ :  $3\ \alpha$ ); 4.74 (broad dd,  $J = 13$  and  $8$  Hz,  $1\text{H}$ : the other H of the  $\text{CH}_2$  in  $5\ \epsilon$ ); 4.84 (mt,  $1\text{H}$ :  $2\ \alpha$ ); 4.89 (dd,  $J = 10$  and  $1$  Hz,  $1\text{H}$ :  $1\ \alpha$ ); 5.29 (dd,  $J = 12$  and  $4.5$  Hz,  $1\text{H}$ :  $4\ \alpha$ ); 5.32 (broad d,  $J = 5.5$  Hz,  $1\text{H}$ :  $5\ \alpha$ ); 5.88 (d,  $J = 9.5$  Hz,  $1\text{H}$ :  $6\ \alpha$ ); 5.90 (mt,  $1\text{H}$ :  $1\ \beta$ ); 6.51 (d,  $J = 10$  Hz,  $1\text{H}$ :  $\text{NH}$  in  $2$ ); 6.99 (broad d,  $J = 8$  Hz,  $1\text{H}$ : aromatic H in the para position with respect to the methylthio); 7.10 and 7.15 (respectively, broad s and broad d,  $J = 8$  Hz,  $1\text{H}$  each: aromatic Hs in the ortho position with respect to the methylthio); from 7.15 to 7.35 (mt,  $6\text{H}$ : aromatic Hs in  $6$  and aromatic Hs in the meta position with respect to the methylthio); 7.43 (broad d,  $J = 8$  Hz,  $1\text{H}$ :  $1'\text{H}_a$ );

7.52 (dd,  $J = 8$  and  $4$  Hz,  $1H: 1'H_5$ ); 7.79 (broad d,  $J = 4$  Hz,  $1H: 1'H_6$ ); 8.38 (d,  $J = 10$  Hz,  $1H: NH$  in 1); 8.73 (d,  $J = 9.5$  Hz,  $1H: NH$  in 6); 11.62 (s,  $1H: OH$ ).

EXAMPLE 30: Preparation of 4 $\epsilon$ -ethoxy-de(4 $\gamma$ -  
5 dimethylamino)pristinamycin I $_A$ .

Strain SP92::pVRC508 is cultured in  
production medium using 60 erlenmeyer flasks, as  
described in Example 3, with 1 ml of a 20 g/l solution  
of (S)-3-O-ethyltyrosine hydrochloride, synthesized as  
10 in Example 37-1, in 0.2N sodium hydroxide solution  
being added at 16h. At the end of 40h of culture, the  
1.8 litres of must recovered from the 60 erlenmeyer  
flasks is extracted with 2 volumes of a mixture  
consisting of 66% 100 mM phosphate buffer, pH 2.9, and  
15 34% acetonitrile, and then centrifuged. The supernatant  
is extracted with 2 times 0.5 volumes of  
dichloromethane. The chloromethylene phases are washed  
with water and then combined, dried over sodium  
sulphate and evaporated. The dry extract is taken up in  
20 20 ml of dichloromethane and injected onto a silica  
(30 g) column which is mounted in dichloromethane and  
eluted successively with plateaus of from 0 to 10%  
methanol in dichloromethane. The fractions containing  
the novel derivative of pristinamycin I $_A$  are combined  
25 and evaporated. 19 mg of dry residue are obtained. The  
latter is taken up in 3 ml of a mixture consisting of  
60% of water and 40% acetonitrile and injected onto a

semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% of acetonitrile. The fractions containing the novel  
 5 pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 15.8 mg of 4 $\epsilon$ -O-ethoxy-de(4 $\gamma$ -dimethyl-amino)pristinamycin I $_A$  are obtained.

10 NMR spectrum.  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm, ref. TMS): 0.55 (dd,  $J = 16$  and  $5.5$  Hz, 1H: 1H of the  $\text{CH}_2$  in 5  $\beta$ ); 0.90 (t,  $J = 7.5$  Hz, 3H:  $\text{CH}_3$  in 2  $\gamma$ ); 1.12 (mt, 1H: 1H of the  $\text{CH}_2$  in 3  $\beta$ ); 1.20 (mt, 1H: 1H of the  $\text{CH}_2$  in 3  $\gamma$ ); 1.31 (d,  $J = 6.5$  Hz, 3H:  $\text{CH}_3$  in 1  $\gamma$ ); 1.49  
 15 (t,  $J = 7$  Hz, 3H:  $\text{CH}_3$  of the ethyl); 1.54 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\gamma$ ); 1.63 and 1.73 (2 mts, 1H each:  $\text{CH}_2$  in 2  $\beta$ ); 2.02 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\beta$ ); 2.22 and 2.33 (respectively, mt and broad d,  $J = 16.5$  Hz, 1H each:  $\text{CH}_2$  in 5  $\delta$ ); 2.46 (d,  $J = 16$  Hz, 1H:  
 20 the other H of the  $\text{CH}_2$  in 5  $\beta$ ); 2.83 (dt,  $J = 13$  and  $4$  Hz, 1H: 1H of the  $\text{CH}_2$  in 5  $\epsilon$ ); 2.95 (dd,  $J = 12$  and  $4$  Hz, 1H: 1H of the  $\text{CH}_2$  in 4  $\beta$ ); 3.22 (mt, 1H: 1H of the  $\text{CH}_2$  in 3  $\delta$ ); 3.27 (s, 3H:  $\text{NCH}_3$ ); 3.39 (t,  $J = 12$  Hz, 1H: the other H of the  $\text{CH}_2$  in 4  $\beta$ ); 3.53 (mt, 1H: the other  
 25 H of the  $\text{CH}_2$  in 3  $\delta$ ); 3.93 and 4.03 (2 mts, 1H each:  $\text{OCH}_2$  of the ethyl); 4.56 (dd,  $J = 7$  and  $5.5$  Hz, 1H: 3  $\alpha$ ); 4.75 (broad dd,  $J = 13$  and  $8$  Hz, 1H: the other H of the  $\text{CH}_2$  in 5  $\epsilon$ ); 4.82 (mt, 1H: 2  $\alpha$ ); 4.88 (dd,  $J = 10$

and 1 Hz, 1H: 1  $\alpha$ ); 5.23 (dd, J = 12 and 4 Hz, 1H: 4  $\alpha$ ); 5.23 (broad d, J = 5.5 Hz, 1H: 5  $\alpha$ ); 5.87 (d, J = 9.5 Hz, 1H: 6  $\alpha$ ); 5.92 (mt, 1H: 1  $\beta$ ); 6.47 (d, J = 10 Hz, 1H: NH in 2); 6.80 (mt, 3H: aromatic H in the ortho and in the para positions with respect to the ethoxy); from 7.10 to 7.35 (mt, 6H: aromatic Hs in 6 and aromatic Hs in the meta position with respect to the ethoxy); 7.43 (dd, J = 8 and 1 Hz, 1H: 1'H<sub>4</sub>); 7.50 (dd, J = 8 and 4 Hz, 1H: 1'H<sub>5</sub>); 7.77 (dd, J = 4 and 1 Hz, 1H: 1'H<sub>6</sub>); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.70 (d, J = 9.5 Hz, 1H: NH in 6); 11.60 (s, 1H: OH).

**EXAMPLE 31: Preparation of 4{-ethylthio-de (4{dimethylamino)pristinamycin I<sub>a</sub>**

Strain SP92::pVRC508 is cultured in production medium using 2 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (S)-4-ethylthiophenylalanine hydrochloride, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 60 ml of must recovered from the 2 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in

20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4 $\zeta$ -ethylthio-de(4 $\zeta$ -dimethylamino)pristinamycin I $_A$  are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4 $\zeta$ -ethylthio-de(4 $\zeta$ -dimethylamino)-pristinamycin I $_A$  are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. ? mg of 4 $\zeta$ -ethylthio-de(4 $\zeta$ -dimethylamino)-pristinamycin I $_A$  are obtained.

NMR spectrum.  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm):  
 0.68 (dd,  $J = 16$  and  $6$  Hz, 1H: 1H of the  $\text{CH}_2$  in 5  $\beta$ );  
 0.92 (t,  $J = 7.5$  Hz, 3H:  $\text{CH}_3$  in 2  $\gamma$ ); from 1.10 to 1.40 (mt, 5H: 1H of the  $\text{CH}_2$  in 3  $\beta$  and 1H of the  $\text{CH}_2$  in 3  $\gamma$  and  $\text{CH}_3$  of the ethyl); 1.32 (d,  $J = 7$  Hz, 3H:  $\text{CH}_3$  in 1  $\gamma$ ); from 1.45 to 1.85 (mt, 3H: the other H of the  $\text{CH}_2$  in 3  $\gamma$  and  $\text{CH}_2$  in 2  $\beta$ ); 2.02 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\beta$ ); 2.18 and 2.37 (respectively, mt and broad d,  $J = 16.5$  Hz, 1H each:  $\text{CH}_2$  in 5  $\delta$ ); 2.45 (broad d,  $J = 16$  Hz, 1H: the other H of the  $\text{CH}_2$  in 5  $\beta$ ); 2.85 (dt,  $J = 13$  and  $4$  Hz, 1H: 1H of the  $\text{CH}_2$  in 5  $\epsilon$ ); 2.90 (mt, 2H:

ArSCH<sub>2</sub> ethyl); 2.98 (dd, J = 12 and 4 Hz, 1H: 1H of the CH<sub>2</sub> in 4 β); 3.25 (s, 3H: NCH<sub>3</sub>); 3.35 (mt, 1H: 1H of the CH<sub>2</sub> in 3 δ); 3.39 (t, J = 12 Hz, 1H: the other H of the CH<sub>2</sub> in 4 β); 3.57 (mt, 1H: the other H of the CH<sub>2</sub> in 3 δ); 4.55 (t, J = 7.5 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 7.5 Hz, 1H, : the other H of the CH<sub>2</sub> in 5 ε); 4.85 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H: 1 α); from 5.25 to 5.40 (mt, 2H: 5 α and 4 α); 5.88 (d, J = 9.5 Hz, 1H: 6 α); 5.91 (mt, 1H: 1 β); 6.55 (d, J = 9.5 Hz, 1H: NH in 2); 7.10 (d, J = 8 Hz, 2H: aromatic Hs in 4 δ); from 7.10 to 7.35 (mt, 7H: aromatic Hs in 6 and 4 ε); 7.44 (limiting AB, 2H: 1'H<sub>4</sub> and 1'H<sub>5</sub>); 7.74 (mt, 1H: 1'H<sub>6</sub>); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.75 (d, J = 9.5 Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

**EXAMPLE 32: Preparation of 4'-ethyl-de(4'-dimethylamino)pristinamycin I<sub>a</sub>**

Strain SP92::pVRC508 is cultured in production medium using 2 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-ethylphenylalanine, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 60 ml of must recovered from the 2 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times [lacuna] volumes of dichloromethane. The



chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is

5 mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4 $\zeta$ -ethyl-de(4 $\zeta$ -dimethyl-amino)pristinamycin I $_A$  are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting

10 of 52% of water and 48% acetonitrile and injected onto a semi-preparative Nucleosil 7 $\mu$  C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4 $\zeta$ -ethyl-

15 de(4 $\zeta$ -dimethylamino)pristinamycin I $_A$  are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 0.50 mg of 4 $\zeta$ -ethyl-de(4 $\zeta$ -dimethylamino)pristinamycin I $_A$  are obtained.

20 NMR spectrum.  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm, ref. TMS): 0.42 (dd,  $J = 16$  and  $5.5$  Hz, 1H: 1H of the  $\text{CH}_2$  in 5  $\beta$ ); 0.92 (t,  $J = 7.5$  Hz, 3H:  $\text{CH}_3$  in 2  $\gamma$ ); from 1.10 to 1.40 (mt, 2H: 1H of the  $\text{CH}_2$  in 3  $\beta$  and 1H of the  $\text{CH}_2$  in 3  $\gamma$ ); 1.23 (t,  $J = 7.5$  Hz, 3H:  $\text{CH}_3$  of the ethyl);

25 1.35 (d,  $J = 7$  Hz, 3H:  $\text{CH}_3$  in 1  $\gamma$ ); from 1.45 to 1.85 (mt, 3H: the other H of the  $\text{CH}_2$  in 3  $\gamma$  and  $\text{CH}_2$  in 2  $\beta$ ); 2.02 (mt, 1H: the other H of the  $\text{CH}_2$  in 3  $\beta$ ); 2.15 and from 2.25 to 2.40 (2 mts, 1H each:  $\text{CH}_2$  in 5  $\delta$ ); from

2.25 to 2.40 (mt, 1H: the other H of the CH<sub>2</sub> in 5 β);  
 2.60 (q, J = 7.5 Hz, 2H: ArCH<sub>2</sub> of the ethyl); 2.83 (dt,  
 J = 13 and 4 Hz, 1H: 1H of the CH<sub>2</sub> in 5 ε); 2.98 (dd, J  
 = 12 and 4 Hz, 1H: 1H of the CH<sub>2</sub> in 4 β); from 3.25 to  
 5 3.35 (mt, 1H: 1H of the CH<sub>2</sub> in 3 δ); 3.27 (s, 3H: NCH<sub>3</sub>);  
 3.39 (t, J = 12 Hz, 1H: the other H of the CH<sub>2</sub> in 4 β);  
 3.59 (mt, 1H: the other H of the CH<sub>2</sub> in 3 δ); 4.58 (dd,  
 J = 7 and 6.5 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and  
 8 Hz, 1H: the other H of the CH<sub>2</sub> in 5 ε); 4.87 (mt, 1H:  
 10 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.24 (broad  
 d, J = 5.5 Hz, 1H: 5 α); 5.29 (dd, J = 12 and 4 Hz, 1H:  
 4 α); 5.88 (d, J = 10 Hz, 1H: 6 α); 5.92 (mt, 1H: 1 β);  
 6.73 (d, J = 10 Hz, 1H: NH in 2); from 7.10 to 7.35  
 (mt, 9H: aromatic Hs in 6 - 4 ε and 4 δ); 7.44 (dd, J =  
 15 8.5 and 1.5 Hz, 1H: 1'H<sub>4</sub>); 7.50 (dd, J = 8.5 and 4.5 Hz,  
 1H: 1'H<sub>5</sub>); 7.80 (dd, J = 4.5 and 1.5 Hz, 1H: 1'H<sub>6</sub>); 8.38  
 (d, J = 10 Hz, 1H: NH in 1); 8.75 (d, J = 10 Hz, 1H: NH  
 in 6); 11.66 (s, 1H: OH).

Using the same fractions derived from the  
 20 silica column described above, which fractions also  
 contain the novel pristinamycin I<sub>x</sub> derivative, 0.3 mg of  
 {*γ*-ethyl-de(4{*γ*-dimethylamino)pristinamycin I<sub>x</sub> is isolated  
 by carrying out semi-preparative column chromatography  
 as described above.

25 NMR spectrum. <sup>1</sup>H (400 MHz, CDCl<sub>3</sub>, δ in ppm):  
 0.04 (mt 1H: 1H of the CH<sub>2</sub> in 5 β); 0.92 (t, J = 7.5 Hz,  
 3H: CH<sub>3</sub> in 2 γ); from 1.10 to 1.40 (mt, 2H: 1H of the

CH<sub>2</sub> in 5  $\delta$  and 1H of the CH<sub>2</sub> in 5  $\gamma$ ); 1.18 (t, J = 7.5 Hz, 3H: CH<sub>3</sub> of the ethyl); 1.30 (d, J = 6.5 Hz, 3H: CH<sub>3</sub> in 1  $\gamma$ ); from 1.45 to 1.85 (mt, 7H: the other H of the CH<sub>2</sub> in 5  $\gamma$  - the other H of the CH<sub>2</sub> in 5  $\delta$  - 1H of the CH<sub>2</sub> in 3  $\beta$  - CH<sub>2</sub> in 3  $\gamma$  and CH<sub>2</sub> in 2  $\beta$ ); 1.81 (broad d, J = 13 Hz, 1H: the other H of the CH<sub>2</sub> in 5  $\beta$ ); 2.02 (mt, 1H: the other H of the CH<sub>2</sub> in 3  $\beta$ ); 2.40 (dt, J = 13 and 4 Hz, 1H: 1H of the CH<sub>2</sub> in 5  $\epsilon$ ); 2.65 (q, J = 7.5 Hz, 2H: ArCH<sub>2</sub> of the ethyl); 2.97 and 3.09 (respectively, dd and t, J = 12 and 5 Hz and J = 12 Hz, 1H each: CH<sub>2</sub> in 4  $\beta$ ); 3.50 and 3.60 (2 mts, 1H each: CH<sub>2</sub> in 3  $\delta$ ); 4.13 (dd, J = 8 and 5 Hz, 1H: 3  $\alpha$ ); 4.49 (broad d, J = 13 Hz, 1H: the other H of the CH<sub>2</sub> in 5  $\epsilon$ ); 4.70 (mt, 2H: 5  $\alpha$  and 4  $\alpha$ ); 4.77 (mt, 1H: 2  $\alpha$ ); 4.83 (dd, J = 10 and 1 Hz, 1H: 1  $\alpha$ ); 5.50 (d, J = 7 Hz, 1H: 6  $\alpha$ ); 5.74 (mt, 1H: 1  $\beta$ ); 6.09 (d, J = 4 Hz, 1H: NH in 4); 6.72 (unres. comp., 1H: NH in 2); 7.07 (d, J = 8 Hz, 2H: aromatic Hs in 4  $\epsilon$ ); 7.15 (d, J = 8 Hz, 2H: aromatic Hs in 4  $\delta$ ); from 7.15 to 7.35 (mt, 5H: aromatic Hs in 6); 7.40 (dd, J = 8 and 1 Hz, 1H: 1'H<sub>4</sub>); 7.45 (dd, J = 8 and 4 Hz, 1H: 1'H<sub>5</sub>); 7.92 (dd, J = 4 and 1 Hz, 1H: 1'H<sub>6</sub>); 8.40 (unres. comp., 1H: NH in 6); 8.50 (d, J = 10 Hz, 1H: NH in 1); 11.72 (s, 1H: OH).

EXAMPLE 33: Preparation of derivatives of phenylalanine and of phenylpyruvic acid which have already been described.

Phenylalanine, and its derivatives

4-methoxyphenylalanine, 4-bromophenylalanine,  
4-chlorophenylalanine, 4-iodophenylalanine,  
4-trifluoromethylphenylalanine, 4-aminophenylalanine  
and 3-methoxyphenylalanine, which are employed in this  
5 work, are commercially available.

The following derivatives of phenylalanine  
can be prepared in accordance with methods described in  
the literature.

(RS)-4-dimethylaminophenylalanine

10 D.F. Elliott, A.T. Fuller, C.R. Harrington,  
J. Chem. Soc., 1948, 85-89.

(RS)-4-diethylaminophenylalanine

Moldaver B.L., Pushkareva Z.V., Zhur.  
Obshchei Khim., 31, 1560-1569 (1961); C.A. 1961,  
15 22226f.; J.A. Stock, J. Chem. Soc, 1959, 90-97

(RS)-4-ethylaminophenylalanine

F. Bergel, J.A. Stock, J. Chem. Soc, 1959,  
90-97.

(RS)-4-phenylphenylalanine

20 J.V. Braun, J. Nelles, Berichte, 66B, 1933,  
1464-1470.

(RS)-4-methylphenylalanine

R.R., Herr, T. Enjoki, J.P. Dailey,  
J. Am. Chem. Soc, 1957, 79, 4229-4231.

25 (RS)-4-methylthiophenylalanine and (R,S)-4-  
ethylthiophenylalanine

R.L. Colescott, R.R. Herr, J.P. Dailey  
J. Am. Chem. Soc, 1957, 79, 4232-4235.

H. Cleland, J. Org. Chem., 1969, 34, 747.

R.R., Herr, T. Enjoki, J.P. Dailey,

(RS)-3,4-dimethylphenylalanine

R.R., Herr, T. Enjoki, J.P. Dailey,

J. Am. Chem. Soc, 1957, 79, 4229-4231.

(RS)-3-trifluoromethylphenylalanine

1.0 hydrochloride

R. Filler and H. Novar. J. Org. Chem, 1960,  
25, 733-736.

(S)-4-aminomethylphenylalanine

G.E. Stokker, W.F. Hoffman and C.F. Homnick,  
15 J. Org. Chem., 1993, 58, 5015-5017.

(R,S)-3-methylphenylalanine

J.H. Burckhalter, V.C. Stephens, J.A.C.S.  
1951, 73, 56-58.

(R,S)-4-acetylphenylalanine

20 J.I. Degaw et coll., J. Med. Che., 1969, 11,  
225-227

(S)-4-O-allyltyrosine

A. Loffet, H. Zang, Int. J. Pept. Protein.  
Res., 1993, 42, 346

25 (S)-4-O-ethyltyrosine

Y. Sasaki et coll., Chem. Pharm. Bull., 1982,  
30, 4435

## (RS)-4-ethylphenylalanine

A. Zhuze et coll., Coll., Czech. Chem.

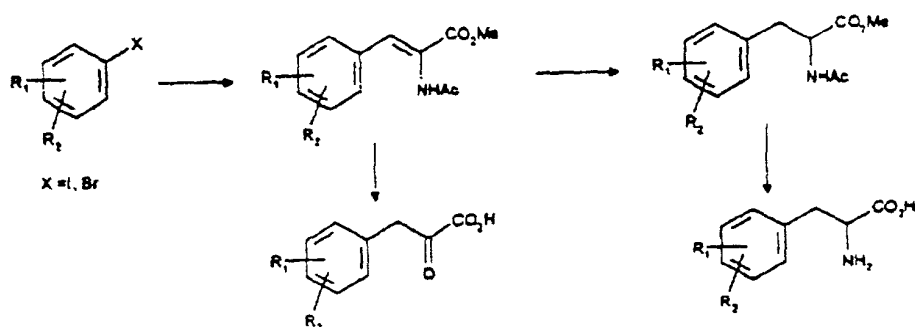
Commmmm., 1965, 62, 2648

4-tert-butylphenylpyruvic acid can be

5 prepared in accordance with R. Breslow, J.W. Canary, M. Varney, S.T. Waddell and D. Yang, J. Am. Chem. Soc., 1990, 112, 5212-5219.

The other derivatives of phenylalanine were prepared in accordance with Examples 34 to 42 which are given below. In these examples, flash chromatography was carried out under a mean nitrogen pressure of 50 kPa using a silica of granule size 40-53  $\mu\text{m}$ , in accordance with Still et al., J. Org. Chem., 43, 2923, (1978).

15 **EXAMPLE 34: Preparation of derivatives of phenylalanine and of a derivative of phenylpyruvic acid using method A.**



34-1 (RS)-4-methylaminophenylalanine, dihydrochloride

20 37 ml of 12 N hydrochloric acid are added to 3.70 g of methyl N-acetyl-4-methylaminophenylalaninate, and the mixture is heated to reflux, while stirring,

for 8 h. After one night at room temperature, the reaction medium is concentrated to dryness under reduced pressure (50 kPa), and the residue is taken up in a mixture of 50 ml of toluene and 50 ml of ethanol, and this mixture is concentrated once again. After drying in a desiccator under reduced pressure (2.6 kPa), 4.18 g (100%) of (RS)-4-methylaminophenylalanine dihydrochloride are obtained in the form of a hygroscopic light beige solid which melts at 158°C.

34-2: Methyl (RS)-N-acetyl-4-methylaminophenylalaninate

0.4 g of 10% palladium on charcoal, and then 50 ml of absolute ethanol, are added to 4 g of methyl 4-methylamino-2-acetamidocinnamate which is placed under a nitrogen atmosphere in an autoclave. The mixture is placed under a pressure of 5.5 bar of hydrogen and heated at 50°C for 15 h with stirring. After stabilizing the temperature at 26°C, and returning the pressure to atmospheric, the medium is filtered through Clarcel®, washed with ethanol and then concentrated to dryness under reduced pressure (2.6 kPa). This results in 3.73 g of methyl N-acetyl-4-methylaminophenylalaninate in the form of white crystals which melt at 118°C.

34-3: Methyl 4-methylamino-2-acetamidocinnamate

5.75 g of methyl 2-acetamidoacrylate, 0.185 g

of palladium acetate, 8.1 g of tetrabutylammonium chloride and 6.03 g of sodium hydrogen carbonate are added to a 3-necked flask which is placed under nitrogen, and then 6.5 g of 4-iodo-N-methylalanine, in solution in 200 ml of DMF, are added to this mixture. The mixture is heated at 82°C for 16 h 30 min and then, after having been cooled down, is poured into 1000 ml of distilled water. The medium is extracted with 250 ml of CH<sub>2</sub>Cl<sub>2</sub>, and the organic phase is separated off; the aqueous phase is then washed twice with 250 ml of CH<sub>2</sub>Cl<sub>2</sub>. The organic phases are combined, dried over sodium sulphate, filtered and concentrated under reduced pressure (50 kPa) at 70°C to yield a brown oil which is purified by flash chromatography (eluent, AcOEt/cyclohexane and then pure AcOEt).

In this way, 4 g of methyl 4-methylamino-2-acetamidocinnamate is obtained in the form of a yellow solid (Merck Silica 5719, R<sub>f</sub> = 0.48), which is employed in this form.

N-Methyl-p-iodoaniline can be prepared in accordance with: S. Krishnamurthy, Tetrahedron Letters, 33, 3315-3318, 1982.

34-4: 4-methylaminophenylpyruvic acid

2.4 g of methyl 4-methylamino-2-acetamidocinnamate and 32 ml of 12 N hydrochloric acid are placed in a round-bottomed flask. The mixture is heated to reflux for 3 h and then cooled down and washed twice with 20 ml of diethyl ether. The aqueous



phase is cooled down to  $-10^{\circ}\text{C}$  and the precipitate which is obtained is filtered and then rinsed with a minimum of cold hydrochloric acid. The solid which is obtained is dried in a desiccator under reduced pressure in order to yield 1.1 g of 4-methylaminophenylpyruvic acid in the form of a light beige solid which melts at  $210^{\circ}\text{C}$ .

34-5: (R,S)-3-Fluoro-4-methylphenylalanine hydrochloride

0.6 g of (R,S)-3-fluoro-4-methylphenylalanine hydrochloride is obtained in the form of white crystals which melt at a temperature greater than  $260^{\circ}\text{C}$  by proceeding as in Example 34-1 but using 1.6 g of methyl N-acetyl(3-fluoro-4-methyl)phenylalaninate.

34-6: Methyl (R,S)-N-acetyl-(3-fluoro-4-methyl)phenylalaninate

1.6 g of methyl N-acetyl-(3-fluoro-4-methyl)phenylalaninate are obtained in the form of a colourless oil (Merck Silica 5719,  $R_f = 0.46$ ; eluent  $\text{CH}_2\text{Cl}_2/\text{AcOEt}$  50/50), by proceeding as in Example 34-2 but using 1.9 g of methyl (4-methyl-3-fluoro)-2-acetamidocinnamate and 0.2 g of 10% palladium on charcoal in 230 ml of ethanol.

34-7: Methyl (3-fluoro-4-methyl)-2-acetamidocinnamate

2.6 g of methyl (3-fluoro-4-methyl)-2-acetamidocinnamate are obtained in the form of a white solid which melts at  $163^{\circ}\text{C}$  by proceeding as in Example

5

hydrochloride

10

15

20

acetamidocinnamate

25

0.14 g of palladium acetate, 6.1 g of tetrabutylammonium chloride, 4.6 g of sodium hydrogen carbonate and 5 g of 4-trifluoromethoxybromobenzene in solution in 150 ml of anhydrous DMF.

5                    34-11: (R,S)-3-Methylthiophenylalanine hydrochloride

1.38 g of (R,S)-3-methylthiophenylalanine hydrochloride are obtained in the form of white crystals which melt at 190°C by proceeding as in  
10 Example 34-1 but using 3.3 g of methyl N-acetyl-3-methylthiophenylalaninate and 40 ml of 12 N hydrochloric acid.

34-12: Methyl (RS)-N-acetyl-3-methylthiophenylalaninate

15                    3.72 g of methyl 3-methylthio-2-acetamidocinnamate, dissolved in 100 ml of methanol, and 30 ml of tetrahydrofuran are placed in a round-bottomed flask, and 1.4 g of magnesium are then added. After reacting for 20 min, the mixture is cooled in an  
20 ice bath and a further 1.4 g of magnesium are then added. The mixture is stirred at room temperature for 18 h and then poured into 1.4 l of distilled water and 300 ml of CH<sub>2</sub>Cl<sub>2</sub>; this mixture is then filtered through Clarcel®. The aqueous phase is adjusted to pH 6 by  
25 adding 12 N hydrochloric acid and then separated off and washed with 100 ml of CH<sub>2</sub>Cl<sub>2</sub>. The organic phases are collected, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure in

order to yield 3.42 g of methyl N-acetyl-3-methylthiophenylalaninate in the form of a colourless oil (Merck Silica 5719,  $R_f=0.5$ ; AcOEt).

34-13: Methyl 3-methylthio-2-

5 acetamidocinnamate

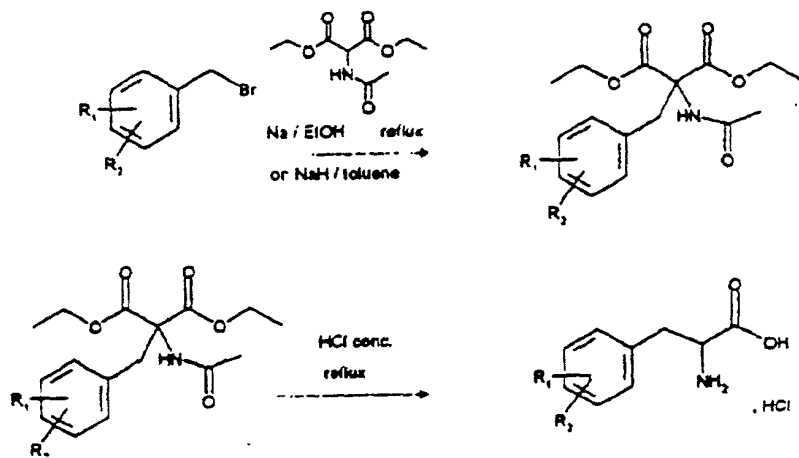
4.8 g of methyl (3-methylthio)-2-acetamidocinnamate are obtained in the form of a white solid which melts at 139°C by proceeding as in Example 34-3 but using 5.6 g of methyl 2-acetamidoacrylate,  
10 0.18 g of palladium acetate, 8.2 g of tetrabutylammonium chloride, 5.86 g of sodium hydrogen carbonate and 6.5 g of 3-iodo-1-methylthiobenzene dissolved in 160 ml of anhydrous DMF.

34-14: 3-Iodomethylthiobenzene

15 20 ml of distilled water and 20 ml of 12 N hydrochloric acid are placed, with stirring, in a three-necked flask, and 10 ml of 3-methylthioaniline are then added using a dropping funnel. The mixture is warmed to ensure dissolution and is then cooled down to  
20 5°C. 5.86 g of sodium nitrite dissolved in 15 ml of water are subsequently added slowly, using a dropping funnel, while maintaining the temperature between 5 and 8°C. 20 min after having completed the addition, 13.57 g of potassium iodide dissolved in 15 ml of water  
25 are added over a period of 10 min and the mixture is then stirred at room temperature for 15 h. The oil which forms is separated from the aqueous phase by decantation, and an aqueous solution of sodium

thiosulphate is then added to it. The aqueous phase is decanted and the product is extracted with 100 ml of dichloromethane. The organic phase is washed with 100 ml of water, and the aqueous phase is adjusted to pH 9 with concentrated sodium hydroxide solution, and then separated off. The organic phase is washed with 2 times 100 ml of water, separated off, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure (50 kPa) at 40°C. The resulting product is purified by flash chromatography (eluent, cyclohexane) in order to yield 13 g of 3-iodo-1-methylthiobenzene in the form of a yellow liquid (Merck Silica 5719,  $R_f=0.8$ /cyclohexane).

**EXAMPLE 35: Preparation of derivatives of phenylalanine using method B.**



**35-1: (RS)-4-tert-butylphenylalanine**

25 g of diethyl 4-(tert-butyl)benzyl acetamidomalonate and 250 ml of 37% hydrochloric acid are added to a three-necked flask which is surmounted

by a condenser. The mixture is stirred and heated to reflux until there is no further evolution of gas. After the reaction medium has been cooled down, the precipitate which is obtained is filtered and then  
5 recrystallized in acetonitrile to yield 25.6 g of (R,S)-4-tert-butylphenylalanine hydrochloride in the form of a white solid which melts at 234°C.

35-2: Diethyl 4-(tert-butyl)benzylacetamidomalonate

10 25 g of 4-(tert-butyl)benzyl bromide, 50 ml of anhydrous toluene and 3.1 g of sodium hydride in 80% suspension in oil are added to a three-necked flask which is surmounted by a condenser, followed by 21.8 g of diethyl acetamidomalonate. The mixture is heated at  
15 110°C for 17 h. After it has been cooled down, 15 ml of absolute ethanol, then 15 ml of 50% ethanol and then 50 ml of water are added slowly to it using a dropping funnel. The organic phase is decanted and the aqueous phase is washed with 3 times 50 ml of diethyl ether.  
20 The organic phases are combined, washed with water and then dried over sodium sulphate. Following filtration and concentration under reduced pressure, the product is crystallized in petroleum ether in order to yield  
25 25 g of diethyl 4-(tert-butyl)benzylacetamidomalonate in the form of a white solid which melts at 80°C.

35-3: (R,S)-3-Methylaminophenylalanine dihydrochloride

1.03 g of a yellow-beige solid are obtained

by proceeding as in Example 35-1 but using 1.17 g of diethyl 3-methylaminobenzylacetamidomalonate and 20 ml of 12 N hydrochloric acid. This yellow-beige solid is dissolved in 20 ml of absolute ethanol, and 0.4 g of animal charcoal is added to this solution. The solution is filtered through Clarcel and then filtered and concentrated under reduced pressure (50 kPa). The same procedure is repeated starting with 1 g of animal charcoal, and the solid which is obtained is triturated in 20 ml of ether. Following filtration and drying under reduced pressure (2.7 kPa) at 50°C, 0.65 g of (R,S)-3-methylaminophenylalanine dihydrochloride is obtained in the form of a white powder which melts at a temperature approaching 135°C (decomposition).

35-4: Diethyl 3-methylaminobenzylacetamidomalonate

3.11 ml of acetic anhydride are placed in a three-necked flask which is maintained under a nitrogen atmosphere. 1.51 ml of formic acid are subsequently added within 3 min at 0°C, and the mixture is then heated at 50°C for 2 hours. The mixture is allowed to return to room temperature, while shaking for 3 h 20 min, and 4 ml of anhydrous THF are added under nitrogen; the mixture is then cooled to -20°C. A solution of 4 g of diethyl 3-aminobenzylacetamidomalonate in a mixture of 15 ml of anhydrous THF and 15 ml of anhydrous dichloromethane is added within 10 min. Stirring is continued for 1 h

10 min at  $-20^{\circ}\text{C}$  and then for 16 h at  $20^{\circ}\text{C}$ . The reaction mixture is concentrated to dryness under reduced pressure (50 kPa) at  $30^{\circ}\text{C}$  and then co-evaporated with 30 ml of anhydrous toluene in order to yield a white solid, which is dissolved in a mixture of 10 ml of anhydrous THF and 20 ml of anhydrous 1,2-dichloroethane, which solution is then placed in a three-necked flask under nitrogen.

The medium is cooled down to  $-5^{\circ}\text{C}$ , and 1.55 ml of borane-dimethyl sulphide complex (2M solution in THF) are then added within 10 min. The mixture is allowed to return to room temperature, and the solution is heated to reflux for 3 h and then stirred at room temperature for 15 h. The reaction medium is cooled to  $0^{\circ}\text{C}$ , and 10 ml of MeOH are then added within 25 min. The mixture is stirred for 45 min at  $0^{\circ}\text{C}$  and then for 30 min at room temperature. It is then cooled to  $0^{\circ}\text{C}$  and HCl gas is bubbled in until a pH of 2 is reached. The mixture is heated at reflux for 1 h and is then concentrated to dryness under reduced pressure at  $30^{\circ}\text{C}$  in order to yield 5 g of a product which is taken up in 30 ml of an aqueous solution of  $\text{NaHCO}_3$  and 30 ml of  $\text{CH}_2\text{Cl}_2$ . The organic phase is decanted and the aqueous phase is washed with 20 ml of water. The organic phases are pooled, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure (2.6 kPa) in order to yield 3.43 g of a yellow oil, which is purified by



flash chromatography (eluent, AcOEt/cyclohexane 50/50). After drying under reduced pressure (2.7 kPa) at 20°C, 1.18 g of diethyl 3-methylaminobenzylacetamidomalonate are thus obtained in the form of a light beige solid which melts at 122°C.

35-5: Diethyl 3-aminobenzylacetamidomalonate

Diethyl 3-aminobenzylacetamidomalonate can be prepared as described in:

T.S. Osdene, D.N. Ward, W.H. Chapman and  
10 H. Rakoff, J. Am. Chem. Soc., 81, 1959, 3100-3102.

35-6: (R,S)-3-Ethylaminophenylalanine  
dihydrochloride

1.7 g of (R,S)-3-ethylaminophenylalanine dihydrochloride are obtained in the form of a  
15 hygroscopic light beige solid, which contains  
10 molar % of (R,S)-3-diethylaminophenylalanine dihydrochloride, by proceeding as in Example 34-1 but using 2 g of ethyl (R,S)-N-acetyl-3-ethylaminophenylalaninate and 30 ml of 12N hydrochloric acid.

20 35-7: (R,S)-N-acetyl-3-ethylaminophenylalaninate

3 g of ethyl (R,S)-N-acetyl-3-aminophenylalaninate, 40 ml of ethanol and 14 g of Raney nickel, which has previously been washed with distilled water  
25 and ethanol, are placed in a round-bottomed flask under a nitrogen atmosphere. The mixture is heated to reflux for 19 h, cooled down, filtered through Clarcel®, and then concentrated to dryness under reduced pressure

(50 kPa) in order to yield 3.07 g of a colourless oil, which is purified by flash chromatography (eluent, AcOEt) in order to yield 2.1 g of ethyl (R,S)-N-acetyl-3-ethylaminophenylalaninate in the form of a colourless oil (Merck Silica 5719,  $R_f=0.6$ : AcOEt) which contains 10% ethyl (R,S)-N-acetyl-3-diethylaminophenylalaninate.

35-8: Ethyl (R,S)-N-acetyl-3-aminophenylalaninate

25 g of a mixture of ethyl (R,S)-N-acetyl-3-nitrophenylalaninate (75 mol %/mol) and diethyl 3-nitrobenzylacetamidomalonate (25 mol %/mol) are placed under nitrogen in an autoclave. 2.5 g of 10% palladium on charcoal and then 200 ml of dichloromethane are added. The mixture is placed under a hydrogen pressure of 9 bar and then stirred at 18°C for 4 h. After returning the pressure to atmospheric, the reaction medium is filtered through Clarcel®, washed with dichloromethane and then concentrated to dryness under reduced pressure (50 kPa) in order to yield a solid, which is recrystallized in 450 ml of distilled water under reflux and in the presence of 4 g of 3S animal charcoal. Following hot filtration through Clarcel®, the mixture is left to crystallize at 4°C, with the crystals being filtered and then dried in order to yield 9.9 g of ethyl (R,S)-N-acetyl-3-aminophenylalaninate in the form of a light beige solid which melts at 106°C and which contains 5% of diethyl 3-aminobenzylacetamidomalonate.

35-9: Ethyl (R,S)-N-acetyl-3-nitrophenyl-  
alaninate and diethyl 3-nitrobenzylacetamidomalonate

600 ml of absolute ethanol and then 7.9 g of sodium are placed, under a nitrogen atmosphere, in a three-necked flask which is surmounted by a condenser. Once dissolution is complete, 74.5 g of diethyl acetamidomalonate and then 60 g of 4-nitrobenzyl chloride in 200 ml of anhydrous ethanol are added. The mixture is heated to reflux for 16 h 30 min. After cooling, the reaction medium is concentrated under reduced pressure (50 kPa) and then taken up in a mixture of 500 ml of  $\text{CH}_2\text{Cl}_2$  and 500 ml of water. The pH is adjusted to 7 by adding 0.5N sulphuric acid, and the organic phase is then separated off and the aqueous phase is washed with 2 times 200 ml of  $\text{CH}_2\text{Cl}_2$ . The organic phases are pooled, washed with 200 ml of water saturated with sodium bicarbonate, separated off and then dried over magnesium sulphate. Following filtration and concentration under reduced pressure (50 kPa), the product is recrystallized in 600 ml of ethanol at reflux in order to yield, after crystallizing at ambient temperature, filtering and drying, 70.4 g of diethyl 3-nitrobenzylacetamidomalonate in the form of white crystals which melt at 156°C. The mother liquors are concentrated and then purified by flash chromatography (eluent, AcOEt) in order to yield 25.6 g of a mixture of ethyl N-acetyl-3-nitrophenylalaninate (75 mol %/mol) and diethyl

3-nitrobenzylacetamidomalonate (25 mol %/mol) in the form of a light beige solid, which is used in this form in the following step.

35-10: (RS,)-3-Dimethylaminophenylalanine  
5 dihydrochloride

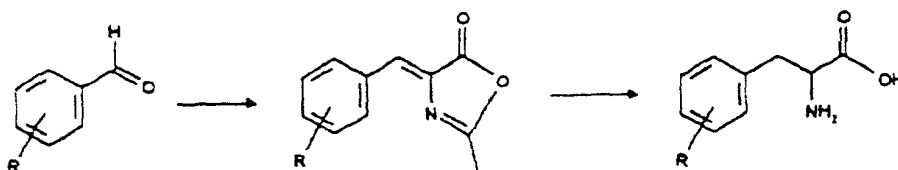
A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 0.72 g of ethyl (RS)-N-acetyl-3-dimethylaminophenylalaninate and 8.6 ml of 10N hydrochloric acid; the solid is subsequently  
10 triturated in 50 ml of acetone, filtered and then dried under reduced pressure (2.7 kPa) at 40°C. 0.68 g (93%) of (RS)-3-dimethylaminophenylalanine dihydrochloride is obtained in the form of a white solid which melts in the region of 120°C (decomposition).

15 35-11: Ethyl (RS)-N-acetyl-3-  
dimethylaminophenylalaninate

4 g of ethyl (RS)-N-acetyl-3-aminophenylalaninate, prepared as described in Example 35-8, in 15 ml of DMF are placed in a three-  
20 necked flask under a nitrogen atmosphere, and 5.5 ml of triethylamine, and then 2.5 ml of methyl iodide and 4 ml of dichloromethane, are added while maintaining the temperature in the region of 30°C using an icebath. The mixture is then warmed at 35°C for 18h. 1 ml of  
25 methyl iodide dissolved in 1 ml of DMF is then added slowly while maintaining the temperature in the region of 30°C; 2.2 ml of triethylamine are then added and the mixture is subsequently warmed for a further 5h at

35°C. The mixture is brought to room temperature and then extracted with 100 ml of ethyl acetate and 150 ml of distilled water. The aqueous phase is separated off after settling and then rewashed with 2 times 70 ml of ethyl acetate. The organic phases are combined, washed with 2 times 80 ml of distilled water and then with 50 ml of distilled water which is saturated with NaCl. The organic phase is separated off after settling, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure in order to yield 2.4 g of a product which is purified by flash chromatography (dichloromethane, MeOH 90/10). 0.72 g (16%) of ethyl (RS)-3-N-acetyl-3-dimethylamino phenylalaninate is thus obtained in the form of yellow crystals.

EXAMPLE 36: Preparation of derivatives of phenylalanine using method C.



36-1: (R,S)-4-Isopropylphenylalanine

7 g of red phosphorus and 8 g of 4-(isopropylbenzylidene)-2-methyl-5-oxazolone, in 47 ml of acetic anhydride, are placed in a three-necked flask, and then 35 ml of 57% hydriodic acid are added slowly, with stirring, using a dropping funnel. Once

the addition is complete, the mixture is heated to reflux for 3 h 30 min and then left at room temperature for 3 days. The reaction mixture is filtered and the solid which is obtained is rinsed twice with 10 ml of acetic acid on each occasion, and the filtrate is then concentrated to dryness under reduced pressure. The residue which is obtained is taken up in 100 ml of distilled water, and this solution is concentrated to dryness under reduced pressure in order to yield a solid which is taken up in 50 ml of distilled water; this solution is then extracted with 3 times 50 ml of diethyl ether after 0.5 g of sodium sulphite have been added. The ether is separated off and the aqueous phase is placed under reduced pressure in order to eliminate traces of diethyl ether. 2 g of animal charcoal are added to the aqueous phase, which is heated at 40-50°C, and then filtered through Clarcel®; rinsing then takes place with a minimum of water. The pH is adjusted to 5 by adding 32% ammonia at 4°C. The precipitate which is obtained is filtered in the cold, rinsed with 2 times 10 ml of water, with 10 ml of ethanol and then with 2 times 10 ml of ether in order to yield, after drying under reduced pressure at 20°C, 3.97 g of (R,S)-4-isopropylphenylalanine in the form of a white solid which melts at a temperature greater than 260°C. (See also Journal of the Takeda Research Laboratories, vol. 43; nos. 3/4, Dec. 1984, pp 53-76).

36-2: 4-(Isopropylbenzylidene)-2-methyl-5-oxazolone

18.52 g of N-acetylglycine, 10.6 g of sodium acetate, 20 ml of 4-isopropylbenzaldehyde and 57 ml of acetic anhydride are placed in a round-bottomed flask which is provided with a condenser. The mixture is stirred for 30 min and then stirred for 1 h at 110°C and subsequently for 15 h at room temperature. The reaction medium is poured into 600 ml of water and 400 ml of petroleum ether which has previously been heated to 50°C. The organic phase is separated off and the aqueous phase is washed with 2 times 150 ml of petroleum ether.

The organic phases are combined, dried over magnesium sulphate, filtered and concentrated under reduced pressure until the volume is 100 ml and a precipitate is obtained. The latter is filtered and washed with 2 times 50 ml of pentane in order to yield 8.2 g of 4-(isopropylbenzylidene)-2-methyl-5-oxazolone in the form of a yellow solid which melts at 77°C.

36-3: (R,S)-4-Butylphenylalanine

0.35 g of (R,S)-4-butylphenylalanine is obtained in the form of a light beige solid which melts at a temperature greater than 260° by proceeding as in Example 36-1 but using 1.49 g of red phosphorus, 1.8 g of 4-(butylbenzylidene)-2-methyl-5-oxazolone, in 9.23 ml of acetic anhydride, and 7.39 ml of 57% hydriodic acid.

36-4: 4-(Butylbenzylidene)-2-methyl-5-oxazolone

1.89 g of 4-(butylbenzylidene)-2-methyl-5-oxazolone are obtained in the form of a yellow solid which melts at 74°C by proceeding as in Example 36-2 but using 8.43 g of N-acetylglycine, 4.92 g of sodium acetate, 9.8 g of 4-butylbenzaldehyde and 26 ml of acetic anhydride.

EXAMPLE 37: Preparation of a derivative of phenylalanine using method D.

37-1: (R,S)-3-Ethoxyphenylalanine hydrochloride (or (R,S)-3-O-ethyltyrosine hydrochloride)

1 g of (R,S)-N-tert-butoxycarbonyl-3-ethoxyphenylalanine, dissolved in 3.6 ml of hydrochloric dioxane, is placed in a round-bottomed flask, and the mixture is then stirred at room temperature for 5 h. The precipitate which forms is filtered, rinsed with dioxane and then ether, and then dried under reduced pressure (2.7 kPa) at 40°C to yield 0.65 g of (R,S)-3-ethoxyphenylalanine hydrochloride in the form of a white solid which melts at 200°C.

37-2: (R,S)-N-tert-Butoxycarbonyl-3-ethoxyphenylalanine

1.33 g of ethyl (R,S)-N-tert-butoxycarbonyl-3-ethoxyphenylalaninate, dissolved in 8 ml of methanol, are placed in a round-bottomed flask, and 8 ml of 1N sodium hydroxide solution are then added. After the



5 mixture has been stirred at room temperature for 18 h,  
it is evaporated under reduced pressure and then  
acidified with 8.56 ml of 1N hydrochloric acid. The  
product is extracted with 2 times 10 ml of ethyl  
acetate, and the organic phases are pooled, washed with  
2 times 10 ml of water, dried, filtered and then  
concentrated to dryness under reduced pressure to yield  
1 g of (R,S)-N-tert-butoxycarbonyl-3-  
ethoxyphenylalanine in the form of a yellow oil (Merck  
10 Silica 5719,  $R_f=0.7$ , eluent: toluene 80/MeOH  
10/diethylamine 10).

37-3: (R,S)-N-tert-Butoxycarbonyl-3-  
ethoxyphenylalaninate

15 1.5 g of (R,S)-N-tert-butoxycarbonyl-3-  
tyrosine, dissolved in 7.5 ml of dry DMF, are placed in  
a three-necked flask under a nitrogen atmosphere, and  
0.508 g of sodium hydride, as a 50% dispersion in oil,  
is then added. After the mixture has been stirred at  
room temperature for 2 h, 0.86 ml of iodoethane is  
20 added and the mixture is then stirred at room  
temperature for 4 h. The medium is filtered and the  
resulting solid is washed with 3 times 10 ml of water  
and then 2 times 10 ml of petroleum ether to yield,  
after drying under reduced pressure (2.7 kPa) at 30°C,  
25 1.33 g of ethyl (R,S)-N-tert-butoxycarbonyl-3-  
ethoxyphenylalaninate in the form of a white solid.

37-4: (R,S)-N-tert-Butoxycarbonyl-3-tyrosine

18 g of (R,S)-3-tyrosine, dissolved in 180 ml

of dioxane, are placed, with stirring, in a three-necked flask, and 99 ml of 1N sodium hydroxide solution, followed by 26 g of di-tert-butyl dicarbonate, dissolved in 160 ml of dioxane, are then added. After the mixture has been stirred for 36 h, it is concentrated under reduced pressure at 30°C and the residue is taken up in 100 ml of distilled water; this solution is acidified to pH 5 with 1N hydrochloric acid and then extracted with 2 times 200 ml of ethyl acetate. The organic phase is dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure at 30°C to yield 30 g of (R,S)-N-tert-butoxycarbonyl-3-tyrosine in the form of a white solid (Merck Silica 5719,  $R_f=0.25$ , eluent: toluene 80, MeOH 10, diethylamine 10).

**EXAMPLE 38: Preparation of derivatives of phenylalanine using method E.**

**38-1: (RS)-4-Diallylaminophenylalanine dihydrochloride**

A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 5.8 g of diethyl 4-diallylaminobenzylacetamido malonate and 48 ml of 10N hydrochloric acid; the solid is then triturated in 50 ml of acetone, filtered, then triturated in 10 ml of dichloromethane, filtered and then rinsed with 3 times 10 ml of ethyl ether. After drying under reduced pressure (2.7 kPa) at 40°C, 4.41 g

of (RS)-4-diallylaminophenylalanine dihydrochloride are obtained in the form of an off-white solid which melts in the region of 135°C (decomposition).

5     38-2: (RS)-4-Allylaminophenylalanine  
      dihydrochloride

      A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 3.27 g of diethyl 4-allylaminobenzylacetamidomalonate and 30 ml of 10N hydrochloric acid; the solid is then triturated  
10    in 50 ml of acetone, filtered and then dried under reduced pressure (2.7 kPa) at 40°C. 2.3 g of (RS)-4-allylaminophenylalanine dihydrochloride are obtained in the form of a white solid which melts in the region of 134°C (decomposition).

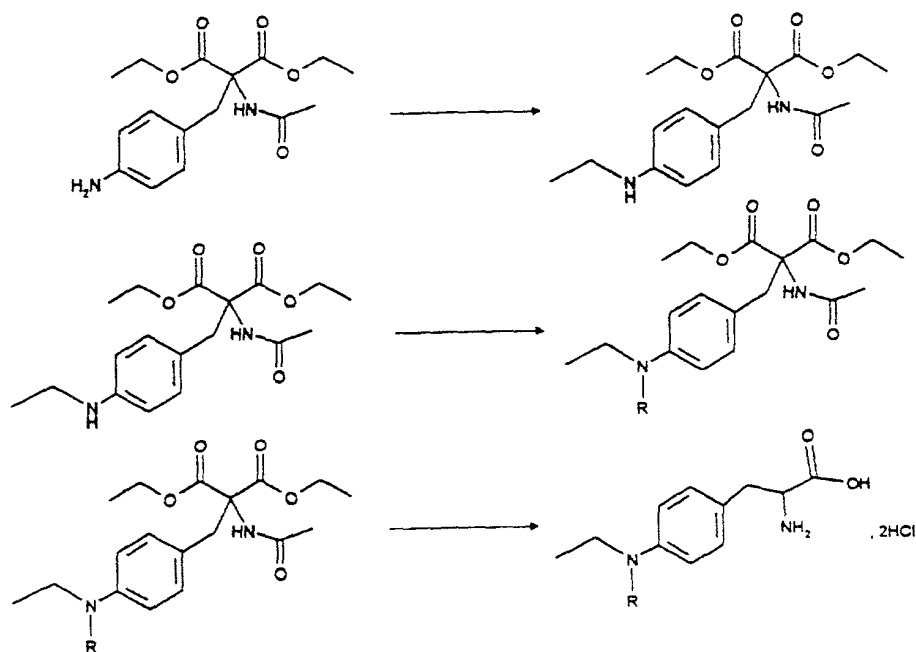
15     38-3: Diethyl 4-diallylaminobenzylacetamido-  
      malonate and diethyl 4-allylaminobenzylacetamido-  
      malonate

      10 g of diethyl 4-aminobenzylacetamido-malonate dissolved in 150 ml of DMF are placed in a  
20    three-necked flask which is surmounted with a dropping funnel and maintained under a nitrogen atmosphere. 6.57 ml of allyl bromide, and then 10.76 ml of triethylamine, are added slowly, at room temperature and while stirring. After stirring for 19h, a further  
25    1.31 ml of allylbromide and 2.15 ml of triethylamine are then added and the mixture is stirred for 26h. The reaction medium is poured onto 1.5 l of distilled water and this mixture is extracted with 1 l of ethyl

acetate. The aqueous phase is separated off after settling and washed with 2 times 500 ml of ethyl acetate. The organic phases are combined, washed with 500 ml of distilled water and then with 500 ml of water which is saturated with sodium chloride, separated off, dried over magnesium sulphate, filtered and then concentrated to dryness in order to yield a chestnut oil; this oil is purified by flash chromatography (eluant,  $\text{CH}_2\text{Cl}_2$  90/AcOEt 10) in order to yield 6.66 g of diethyl 4-diallylaminobenzylacetamidomalonate in the form of a beige solid which melts at 94-96°C ( $R_f$  = 0.6, AcOEt 50/cyclohexane 50) and 3.49 g of diethyl 4-allylaminobenzylacetamidomalonate in the form of a beige solid which melts at 104-106°C ( $R_f$  = 0.45 AcOEt 50/cyclohexane 50).

The diethyl 4-aminobenzylacetamidomalonate can be prepared as described in J.B. Burckhalter, VC Stephens, J. Am. Chem. Soc. 56, 1951, 73.

**EXAMPLE 39: Preparation of derivatives of phenylalanine using method F**



**39-1: (RS)-4-ethylisopropylphenylalanine dihydrochloride**

5 A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 2.9 g of diethyl 4-ethylisopropylbenzylacetamidomalonate and 24.6 ml of 10N hydrochloric acid; the solid is then trituated in 20 ml of acetone, filtered and then dried

10 under reduced pressure (2.7 kPa) at 40°C. 2 g of (RS)-4-ethylisopropylaminophenylalanine dihydrochloride are obtained in the form of a white solid which melts in the region of 147°C (decomposition).

39-2: Diethyl 4-ethylisopropylaminobenzyl-  
acetamidomalonate

15 g of diethyl 4-ethylaminobenzylacetamido-  
malonate in 70 ml of THF are placed in a three-necked  
5 flask which is maintained under a nitrogen atmosphere;  
6.4 ml of 2-iodopropane, and then 8.4 ml of 1,5-  
diazabicyclo[4.3.0]non-5-ene are added and the mixture  
is then heated at 60°C for 24h. 2.13 ml of 2-  
iodopropane, and then 8.4 ml of 1,5-  
10 diazabicyclo[4.3.0]non-5-ene, are subsequently added  
and the mixture is then heated for a further 24h at  
60°C. The mixture is brought to room temperature and  
then extracted with 50 ml of dichloromethane and 50 ml  
of distilled water. The aqueous phase is separated off  
15 after settling and then rewashed with 2 times 30 ml of  
dichloromethane. The organic phases are combined,  
washed with 60 ml of distilled water and then with  
50 ml of distilled water which is saturated with NaCl.  
The organic phase is separated off after settling,  
20 dried over magnesium sulphate, filtered and then  
concentrated to dryness under reduced pressure in order  
to yield 16.2 g of a product which is purified by flash  
chromatography (dichloromethane, MeOH 90/10). This  
results in 4.59 g of a product which is recrystallized  
25 in 45 ml of cyclohexane in order to yield 3.44 g of  
diethyl 4-ethylisopropylaminobenzylacetamidomalonate in  
the form of white crystals which melt at 80°C.

39-3: Diethyl 4-ethylaminobenzylacetamido-  
malonate

Diethyl 4-ethylaminobenzylacetamidomalonate  
can be prepared by proceeding as in Example 35-7 but  
5 using 22 g of diethyl 4-aminobenzylacetamidomalonate,  
500 ml of ethanol and 70 g of Raney nickel. This  
results in 23.8 g of diethyl 4-ethylaminobenzyl-  
acetamidomalonate in the form of an off-white solid  
which melts at 136°C.

10 39-4: (RS)-4-Allylethylaminophenylalanine  
dihydrochloride

A solid is obtained, after evaporation, by  
proceeding as in Example 35-1 but using 4.54 g of  
diethyl 4-allylethylbenzylacetamidomalonate and 37.9 ml  
15 of 10N hydrochloric acid; the solid is then dried under  
reduced pressure (2.7 kPa) at 40°C. 3.67 g of (RS)-4-  
allylethylaminophenylalanine dihydrochloride are  
obtained in the form of a brown solid which melts in  
the region of 130°C (decomposition).

20 39-5: Diethyl 4-allylethylaminobenzyl-  
acetamidomalonate

5.6 g of a solid are obtained, after  
purification by flash chromatography (eluant, CH<sub>2</sub>Cl<sub>2</sub>/  
AcOET 90-10 by volume), by proceeding as in Example  
25 39-2 but using 8 g of diethyl 4-ethylaminobenzyl-  
acetamidomalonate, 4 ml of allyl bromide and 5.82 ml of  
1,5-diazabicyclo[4.3.0]non-5-ene in 50 ml of THF; the  
solid is then recrystallized in 35 ml of cyclohexane.

This results in 5.43 g of diethyl 4-allylethylamino-benzylacetamidomalonate in the form of a white solid which melts at 86°C.

39-6: (RS)-4-Ethylpropylaminophenylalanine  
5 dihydrochloride

A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 2.5 g of diethyl 4-ethylpropylaminobenzylacetamidomalonate and 21 ml of 10N hydrochloric acid;. The solid is then  
10 dried under reduced pressure (2.7 kPa) at 40°C. 2 g (97%) of (RS)-4-ethylpropylaminophenylalanine dihydrochloride are obtained in the form of a white solid which melts in the region of 147°C (decomposition).

15 39-7: Diethyl 4-ethylpropylaminobenzyl-acetamidomalonate

2.8 g of a solid are obtained, after reacting for 36 hours and then purifying by flash chromatography (eluant, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 97-3 by volume), by proceeding as  
20 in Example 39-2 but using 10 g of diethyl 4-ethylamino-benzylacetamidomalonate, 5.6 ml of 1-iodopropane and 7.2 ml of 1,5-diazabicyclo[4.3.0]non-5-ene in 70 ml of THF; the solid is then recrystallized in 26 ml of cyclohexane. This results in 2.9 g of diethyl 4-ethyl-  
25 propylaminobenzylacetamidomalonate in the form of a white solid which melts at 84-86°C.



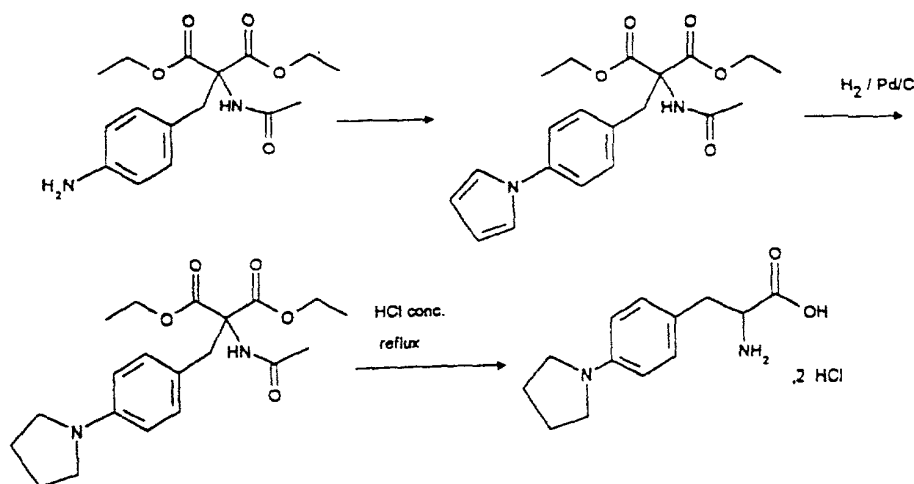
39-8: (RS)-4-Ethylmethylcyclopropylamino-phenylalanine dihydrochloride

A solid is obtained, after reacting for 3 days and then evaporating, by proceeding as in Example 35-1 but using 3 g of diethyl 4-ethylmethylcyclopropylaminobenzylacetamidomalonate and 25 ml of 10N hydrochloric acid; the solid is then triturated in 40 ml of acetone, filtered and then dried under reduced pressure (2.7 kPa) at 40°C. 2.24 g of (RS)-4-ethylmethylcyclopropylaminophenylalanine dihydrochloride are obtained in the form of a white solid which melts in the region of 140°C (decomposition).

39-9: Diethyl 4-ethylmethylcyclopropylamino-benzylacetamidomalonate

By proceeding as in Example 39-2, but using 8 g of diethyl 4-ethylaminobenzylacetamidomalonate, 2.6 ml of bromomethylcyclopropane and 2.97 ml of 1,5-diazabicyclo[4.3.0]non-5-ene in 50 ml of THF, 3.3 g of diethyl 4-ethylmethylcyclopropylaminobenzylacetamidomalonate are obtained, after reacting for 3 days and then purifying by flash chromatography (eluant CH<sub>2</sub>Cl<sub>2</sub>/AcOEt 90-10 by volume), in the form of a white solid which melts at 112-114°C.

**EXAMPLE 40: Preparation of derivatives of phenylalanine using method G**



**40-1: (RS)-4-(1-Pyrrolidinyl)phenylalanine dihydrochloride**

5 A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 1.5 g of diethyl 4-(1-pyrrolidinyl)benzylacetamidomalonate and 40 ml of 5N hydrochloric acid; the solid is then trituated in 15 ml of acetone, filtered and then dried  
10 under reduced pressure (2.7 kPa) at 40°C. 0.6 g of (RS)-4-(1-pyrrolidinyl)phenylalanine dihydrochloride is obtained in the form of an off-white solid.

**40-2: Diethyl 4-(1-pyrrolidinyl)benzylacetamidomalonate**

15 4 g of diethyl 4-(1-pyrrolyl)benzylacetamidomalonate, dissolved in 100 ml of MeOH, and 1 g of 10% palladium on charcoal are placed in an autoclave. After having purged the autoclave 3 times with nitrogen, the

product is hydrogenated at 19°C under a pressure of 14 bars of hydrogen. After stirring for 25 hours, the hydrogenation is stopped and the product is filtered through Clarcel® and rinsed with dichloromethane; the solution is then concentrated under reduced pressure in order to yield 3.85 g of a solid which is triturated in a mixture of 50 ml of heptane and 10 ml of ethyl ether. The resulting solid is filtered, dried and then purified by flash chromatography (eluant CH<sub>2</sub>Cl<sub>2</sub>/acetone 90/10 by volume) in order to yield 1.6 g of diethyl 4-(1-pyrrolidiny1)benzylacetamidomalonate in the form of a white solid which melts at 132°C.

40-3: Diethyl 4-(1-pyrrolyl)benzylacetamido-malonate

4,6 g of diethyl 4-aminobenzylacetamidomalonate in 104 ml of acetic acid are placed in a three-necked flask which is maintained under nitrogen. 7.02 g of sodium acetate are added, followed by 1.87 ml of 2,5-dimethoxytetrahydrofuran. The mixture is heated at 65°C for 1h 15 min, then cooled down and extracted with 100 ml of dichloromethane and 100 ml of distilled water. The aqueous phase is separated off after settling and then washed with 3 times 100 ml of dichloromethane. The organic phases are combined, washed with 100 ml of water and then with 100 ml of a saturated solution of NaCl, separated off after settling and then dried over magnesium sulphate; the phases are filtered and then evaporated to dryness

under reduced pressure (50 kPa) in order to yield 6.2 g of a solid which is purified by flash chromatography (eluent CH<sub>2</sub>Cl<sub>2</sub>/acetone 75/25 by volume). This results in 3.57 g of diethyl 4-(1-pyrrolyl)benzylacetamido-  
5 malonate in the form of a beige solid which melts at 110°C.

**EXAMPLE 41: Preparation of derivatives of phenylalanine using method H**

**41-1: (RS)-4-Ethylthiomethylphenylalanine**

10 300 ml of anhydrous methanol are placed in a three-necked flask which is maintained under nitrogen; subsequently, 1.72 g of sodium methoxide, and then 5.55 ml of ethyl mercaptan, are added while stirring. The solvent is concentrated under reduced pressure at  
15 40°C in order to yield 8.5 g of the sodium salt of ethyl mercaptan, which is dissolved in 100 ml of anhydrous THF. 3.6 g of (RS)-4-chloromethylphenylalanine are added at room temperature and the mixture is then heated to reflux for 18h. The solvent is  
20 evaporated under reduced pressure at 40°C and the residue is taken up in 100 ml of distilled water. The turbid solution which is obtained is acidified with 5 ml of acetic acid. The resulting precipitate is filtered, rinsed with distilled water and then dried at  
25 60°C under reduced pressure in order to yield 3.6 g of a solid which is purified by flash chromatography (eluant AcOEt 60, AcOH 12, water 10). This results in

256 mg of (RS)-4-ethylthiomethylphenylalanine in the form of a white solid which melts at 251°C.

The (RS)-4-chloromethylphenylalanine can be obtained by analogy with (S)-4-

- 5 chloromethylphenylalanine as described in: R.Gonzalez-Muniz, F. Cornille, F. Bergeron, D. Ficheux, J. Pothier, C. Durieux and B. Roques, Int. J. Pept. Protein. Res., 1991, 37 (41), 331-340.

10 **EXAMPLE 42: Preparation of derivatives of phenylalanine using method I**

42-1: (S)-4-O-(2-Chloroethyl)tyrosine hydrochloride

- 5 g of (S)-N-tert-butoxycarbonyl-4-O-(2-chloroethyl)tyrosine, dissolved in 50 ml of  
15 hydrochloric dioxane, are placed in a round-bottomed flask. After having been stirred for 28h, the mixture is concentrated to dryness under reduced pressure. The resulting residue is taken up in 50 ml of ether and this solution is then stirred and filtered. The  
20 resulting solid is washed with 2 times 25 ml of ether and then dried under reduced pressure in order to yield 1.58 g of (S)-4-O-(2-chloroethyl)tyrosine hydrochloride in the form of a white solid which melts at 260°C.

- 42-2: (S)-N-tert-Butoxycarbonyl-4-O-(2-chloroethyl)tyrosine  
25

14 g of (S)-N-tert-butoxycarbonyltyrosine, dissolved in 140 ml of DMF, are placed in a three-

necked flask under a nitrogen atmosphere. 4.8 g of 50% sodium hydride in oil are added slowly using a spatula. 16.87 g of 1-tosyl-2-chloroethanol are added after the mixture has been stirred for 2h at room temperature.

- 5 2.4 g of 50% sodium hydride in oil, and a further 8.4 ml of 1-tosyl-2-chloroethanol, are added after the mixture has been stirred for 2 days. The same procedure is carried out after 24h and the stirring is continued for a further 24h. The reaction is stopped by adding
- 10 100 ml of distilled water, and the reaction mixture is concentrated to dryness under reduced pressure. The residue which is obtained is taken up in 100 ml of distilled water and then extracted with 3 times 100 ml of ethyl acetate. The aqueous phase is separated off
- 15 after settling and acidified to pH3 with 50 ml of 1N HCl, and the product is extracted with 3 times 100 ml of ethyl acetate. The organic phases are combined, washed with 2 times 50 ml of water, separated off, dried over magnesium sulphate, filtered and then
- 20 concentrated to dryness under reduced pressure in order to yield 13.51 g of (S)-N-tert-butoxycarbonyl-4-O-(2-chloroethyl)tyrosine in the form of a chestnut oil (Rf = 0.5, toluene 70%/methanol 20%/diethylamine 10%), which is used as such in the following step.

TABLE V

	MICROORGANISMS	ANTIBIOTICS
	<b>FUNGI</b>	
	<u>Micromonospora</u> sp.	Vernamycins
5	<b>STREPTOMYCES</b>	
	<u>S. alborectus</u>	Virginiamycins
	<u>S. conganensis</u> (ATCC13528)	F1370 A, B
	<u>S. diastaticus</u>	Plauracins, Streptogramins
	<u>S. graminofasciens</u>	Streptogramins
10	<u>S. griseus</u> (NRRL2426)	Viridogrisein (Etamycin)
	<u>S. griseoviridus</u>	Griseoviridin
	<u>S. griseoviridus</u> (FERMP3562)	Neoviridogriseins
	<u>S. lavendulae</u>	Etamycins
	<u>S. loidensis</u> (ATCC11415)	Vernamycins
15	<u>S. mitakaensis</u> (ATCC15297)	Mikamycins
	<u>S. olivaceus</u> (ATCC12019)	Synergistins (PA 114 A, B)
	<u>S. ostreogriseus</u> (ATCC27455)	Ostreogrycins
	<u>S. pristinaespiralis</u> (ATCC25486)	Pristinamycins
	<u>S. virginiae</u> (ATCC13161)	Virginiamycins (Staphylomycins)
20	<b>ACTINOMYCETES</b>	
	<u>A. auranticolor</u> (ATCC31011)	Plauracins
	<u>A. azureus</u> (ATCC31157)	Plauracins
	<u>A. daghestanicus</u>	Etamycin
	<u>A. philippinensis</u>	A-2315 A,B,C
25	<u>Actinoplanes</u> sp. (ATCC3302)	A15104
	<u>Actinoplanes</u> sp.	A17002 A,B,C,F
	<u>Actinomadura flava</u>	Madumycins

Abbreviations employed:

	AcOEt	ethyl acetate
	DNA	deoxyribonucleic acid
	AMP	adenosine 5'-monophosphate
5	HPLC	high-performance liquid chromatography
	dCTP	deoxycytosine 5'-triphosphate
	DMF	dimethylformamide
	DMPAPA	4-dimethylamino-L-phenylalanine
	HCl	hydrochloric acid
10	HT7	Hickey Tresner solid medium
	3-HPA	3-hydroxypicolinic acid
	IPTG	isopropyl- $\beta$ -D-thiogalactopyranoside
	kb	kilobase
	LB	Luria broth (rich growth medium for
15		<u>E. coli</u> )
	MeOH	methanol
	MMPAPA	4-methylamino-L-phenylalanine
	NaOH	sodium hydroxide
	PAPA	4-amino-L-phenylalanine
20	PEG	polyethylene glycol
	P I	pristinamycin I
	P II	pristinamycin II
	bp	base pair
	SAM	S-adenosylmethionine
25	TE	10 mM Tris-HCl buffer, 1 mM EDTA, pH 7.5
	THF	tetrahydrofuran
	Tris	2-amino-2-(hydroxymethyl)-1,3-



[illegible][illegible]

- [illegible]

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England.

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SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

- 5 (A) NAME: RHONE-POULENC RORER S.A.  
(B) STREET: 20, avenue Raymond ARON  
(C) CITY: ANTONY  
(E) COUNTRY: FRANCE  
(F) POSTAL CODE: 92165

10 (ii) TITLE OF INVENTION: NOVEL STREPTOGRAMINS  
AND PROCESS FOR PREPARING STREPTOGRAMINS BY  
MUTASYNTHESIS.

(iii) NUMBER OF SEQUENCES: 8

## (iv) COMPUTER READABLE FORM:

- 15 (A) MEDIUM TYPE: Tape  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: Release #1.0, Version  
#1.25 (OEB)

## (2) INFORMATION FOR SEQ ID NO: 1:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2888 base pairs  
(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

5 (iii) ANTISENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Streptomyces

*pristinaespiralis*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

10	20	30	40	50	60
CTGCAGTTCC	CCGGGGCCAC	CGTGCTCAGC	TCCTCACC CG	AACGGTTCCT	GCGCATCGGC
70	80	90	100	110	120
GCGGACGGCT	GGGCGGAGTC	CAAACCCATC	AAGGGCACCC	GCCCCCGCGG	CGCCGGCCCC
130	140	150	160	170	180
GCCCAGGACG	CCGCCGTCAA	GGCCTCCCTC	GCCGCGGCGG	AGAAGGACCG	CAGCGAGAAC
190	200	210	220	230	240
CTGATGATCG	TCGACCTGGT	CCGCAACGAC	CTCGGCCAGG	TCTGCGACAT	CGGCTCCGTC
250	260	270	280	290	300
CACGTACCGG	GCCTGTTCGA	GGTGGAGACC	TACGCCACCG	TCCACCAGCT	CGTCAGCACC
310	320	330	340	350	360
GTCCGCGGCC	GCCTGGCGGC	CGACGTCTCC	CGCCCCCGCG	CGGTACGGGC	CGCCTTCCCC

370	380	390	400	410	420
GGCGGGTCGA	TGACCGGGCG	GCCCAAGGTC	CGCACCATGC	AGTTCATCGA	CCGGCTCGAG
430	440	450	460	470	480
AAGGGCCCGC	GCGGCGTGTA	CTCGGGCGCG	CTGGGCTACT	TCGCCCTCAG	CGGCGCGGCC
490	500	510	520	530	540
GACCTCAGCA	TCGTTCATCCG	CACCATCGTC	GCCACCGAGG	AGGCCGCCAC	CATCGGCGTG
550	560	570	580	590	600
GGCGGCGCGG	TCGTGCGCCT	GTCCGACCCC	GACGACGAGG	TCCGCGAAAT	GCTCCTCAAG
610	620	630	640	650	660
GCGCAGACCA	CCCTCGCCCG	CCTGCGCCAG	GCACACGCGG	GCGCCACCGC	CTCGGACCGT
670	680	690	700	710	720
GAACTCCTGG	CCGGCAGCCT	GCGGTGACCC	ACCCACCGCC	CCACCCCGGC	CACCGCAACC
730	740	750	760	770	780
CCGGCTCACC	CCCGGGGCGG	CCGCGCGCGG	TGCCGCCC GG	CGGCCGACCC	GGCGACGGGT
790	800	810	820	830	840
CCGCTCGCGG	ACCGGGTGAC	GGACCCGGCG	GCGGGGCGCG	CGGCGGGCCG	GGACGTGGGC
850	860	870	880	890	900
CGGGACGTGG	GCCCGGCGTC	CCCGGCGACC	GGCACGGCGG	CGGGCCCCGA	CGTGGGCCCC
910	920	930	940	950	960
GCGTGCCCGG	CGACCGGCAC	GGTGGCGGGG	CGGGGCGGGG	GACGGTCAGT	GCAGGGCGGT
970	980	990	1000	1010	1020
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1270	1280	1290	1300	1310	1320
CAGCCCCAAG	GCCAGCACCG	CGGCATGCGT	GGCGGCCTGC	TGCGCGGGCG	TCAGCTCGTC
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CAGCGAGGGG	GCGAACATCG	GGTTCAGCCC	CACCGCCTGC	AGCCCCGGCG	CCGCCTCACG
1510	1520	1530	1540	1550	1560
CAGCCGCCCG	GCGATCCGGC	TCTTGACCGA	CAAGGTGTCC	GCGAGCACCG	CACCGGGCCG
1570	1580	1590	1600	1610	1620
CATCACCCCC	GCCAGCACCT	CCACCGCCTC	CCACGCCACC	GGCTCCGGCA	CCGCCAGCAC
1630	1640	1650	1660	1670	1680
CACCACGTCC	GCCGCCGCCA	GCGCCGCGAC	CGCCTCCGGC	CCCGGCCGCC	GCACATCACC
1690	1700	1710	1720	1730	1740

GGCCACCACC CGCACCCTGT CCGCCCGCACC GGCCCCGGCC ACGTCCAGCC AGGTCACCGC  
1750 1760 1770 1780 1790 1800  
CACCCTCCGAA CGCACCAGCC AGTGGCTGAA CATGCGGCCC ACCGCACCGG CCCCCCCCAC  
1810 1820 1830 1840 1850 1860  
CACCACACAA CGCCCGAACA CCGAACCACC CCTCATCCGC GTTCCCGATC CCCCCGGTAC  
1870 1880 1890 1900 1910 1920  
GGAGGAAGAA CCATGACCCC GCCCGCCATC CCCGCGGCCC CGCCCGCCAC CGGGCCCGCC  
1930 1940 1950 1960 1970 1980  
CCCGCCACCG ACCCCCTCGA CGCGCTGCGC GCCCGCCTGG ACGCCGCGGA CGCCGCCCTG  
1990 2000 2010 2020 2030 2040  
CTGGACGCGG TCCGCACACG CCTGGACATC TGCCTGCGCA TCGGCGAGTA CAAGCGCCTC  
2050 2060 2070 2080 2090 2100  
CACCAGGTGC CGATGATGCA GCCCCACCGG ATCGCCCAGG TCCACGCCAA CGCCGCCCGC  
2110 2120 2130 2140 2150 2160  
TACGCGGCGG ACCACGGCAT CGACCCCGCC TTCCTGCGCA CCCTGTACGA CACGATCATC  
2170 2180 2190 2200 2210 2220  
ACCGAGACCT GCGCCTCGA GGACGAGTGG ATCGCCTCCG GCGGCGCCCC CGTCCCCACG  
2230 2240 2250 2260 2270 2280  
CCCGTGACG CGTCCGCGTC CGCGCGGGGG GCCGTGTCTG GACCGCCGCC GCACCCACCC  
2290 2300 2310 2320 2330 2340  
TCGCCCAGGC GCTGGACGAG GCCACCGGGC AGCTGACCGG CGCCGGGATC ACCGCCGACG  
2350 2360 2370 2380 2390 2400  
CCGCCCCGGG CGACACCCGG CTGCTGGCCG CCCACGCCTG CCAGGTCGCC CCGGGGGACC  
2410 2420 2430 2440 2450 2460  
TCGACACCTG CCTGGCCGGC CCGGTGCCGC CCCGTTCTG GCACTACGTC CGGCGCCGTC  
2470 2480 2490 2500 2510 2520  
TGACCCGCGA ACCCGCCGAA CGCATCGTCG GCCACGCCTA CTTCATGGGC CACCGCTTCG  
2530 2540 2550 2560 2570 2580  
ACCTGGCCCC CGGCGTCTTC GTCCCCAAAC CCGAGACCGA GGAGATCACC CGGGACGCCA  
2590 2600 2610 2620 2630 2640  
TCGCCCCGCT GGAGGCCCTC GTCCGCGCGG GCACCACCGC ACCCCTGGTC GTCGACCTGT  
2650 2660 2670 2680 2690 2700  
GCGCCGGACC GGGCACCATG GCCGTCAACC TGGCCCGCCA CGTACCGGCC GCCCGCGTCC  
2710 2720 2730 2740 2750 2760  
TGGGCATCGA ACTCTCCAG GCCCGCGCCC GCGCCGCCC GCGCAACGCC CGCGGCACCG  
2770 2780 2790 2800 2810 2820  
GCGCCCGCAT CGTGCAGGGC GACGCGCGCG ACGCCTTCCC CGAACTGAGC GGCACCGTCG  
2830 2840 2850 2860 2870 2880  
ACCTCGTCGT CACCAACCCG CCCTACATCC CCATCGGACT GCGCACCTCC GCACCCGAAG  
TGCTCGAG

(3) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 888 base pairs
- (B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

5 (iv) ANTISENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Streptomyces  
pristinaespiralis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2

ATG AGG GGT GGT TCG GTG TTC GGG CGT TGT GTG GTG GTG GGC GGG GCC GGT GCG	54
Met Arg Gly Gly Ser Val Phe Gly Arg Cys Val Val Val Gly Gly Ala Gly Ala	18
GTG GGC CGC ATG TTC AGC CAC TGG CTG CTG CGT TCG GGG GTG GCG GTG ACC TGG	108
Val Gly Arg Met Phe Ser His Trp Leu Val Arg Ser Gly Val Ala Val Thr Trp	36
CTG GAC GTG GCC GGG GCC GGT GCG GCG GAC GGG GTG CCG GTG GTG GCC GGT GAT	162
Leu Asp Val Ala Gly Ala Gly Ala Ala Asp Gly Val Arg Val Val Ala Gly Asp	54
GTG CCG CCG CCG GGG CCG GAG GCG GTC GCG GCG CTG GCG GCG GCG GAC GTG GTG	216
Val Arg Arg Pro Gly Pro Glu Ala Val Ala Ala Leu Ala Ala Ala Asp Val Val	72
GTG CTG GCG GTG CCG GAG CCG GTG GCG TGG GAG GCG GTG GAG CTG CTG GCG GGG	270
Val Leu Ala Val Pro Glu Pro Val Ala Trp Glu Ala Val Glu Val Leu Ala Gly	90
GTG ATG CCG CCC GGT GCG GTG CTC GCG GAC ACC TTG TCG GTC AAG AGC CCG ATC	324
Val Met Arg Pro Gly Ala Val Leu Ala Asp Thr Leu Ser Val Lys Ser Arg Ile	108
GCC GGG CCG CTG CGT GAG GCG GCG CCG GGG CTG CAG GCG GTG GGG CTG AAC CCG	378
Ala Gly Arg Leu Arg Glu Ala Ala Pro Gly Leu Gln Ala Val Gly Leu Asn Pro	126
ATG TTC GCC CCC TCG CTG GGT CTT CAG GGG CCG CCG GTG GCG GCG GTG GTG GTC	432
Met Phe Ala Pro Ser Leu Gly Leu Gln Gly Arg Pro Val Ala Ala Val Val Val	144
ACC GAC GGG CCC GGT GTG CCG GCC CTG GTG GAG CTG GTG GCC GGG TGG GGG GCC	486
Thr Asp Gly Pro Gly Val Arg Ala Leu Val Glu Leu Val Ala Gly Trp Gly Ala	162
CGG GTG GTG GAG ATG CCG GCG CCG CCG CAC GAC GAG CTG ACC GCC GCG CAG CAG	540
Arg Val Val Glu Met Pro Ala Arg Arg His Asp Glu Leu Thr Ala Ala Gln Gln	180
GCC GCC ACG CAT GCC GCG GTG CTG GCC TTC GGG CTG GCG CTG GGT GA CTG TCG	594
Ala Ala Thr His Ala Ala Val Leu Ala Phe Gly Leu Gly Leu Gly Glu Leu Ser	198

T05011-443600

5

10

(ii) MOLECULE TYPE: cDNA

(iii) ANTISENSE: no

## pristinaespiralis

(A) ORGANISM: Streptomyces



## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3

ATG ACC CCG CCC GCC ATC CCC GCG GCG CCG CCC GCC ACC GGG CCC GCC CCC GCC	54
Met Thr Pro Pro Ala Ile Pro Ala Ala Pro Pro Ala Thr Gly Pro Ala Ala Ala	18
ACC GAC CCC CTC GAC GCG CTG CGC GCC CGC CTG GAC GCC GCG GAC GCC GCC CTG	108
Thr Asp Pro Leu Asp Ala Leu Arg Ala Arg Leu Asp Ala Ala Asp Ala Ala Leu	36
CTG GAC GCC GTC CGC ACA CGC CTG GAC ATC TGC CTG CGC ATC GGC GAG TAC AAG	162
Leu Asp Ala Val Arg Thr Arg Leu Asp Ile Cys Leu Arg Ile Gly Glu Tyr Lys	54
CGC CTC CAC CAG GTG CCG ATG ATG CAG CCC CAC CGG ATC GCC CAG GTC CAC GCC	216
Arg Leu His Gln Val Pro Met Met Gln Pro His Arg Ile Ala Gln Val His Ala	72
AAC GCC GCC CGC TAC GCC GCC GAC CAC GGC ATC GAC CCC GCC TTC CTG CGC ACC	270
Asn Ala Ala Arg Tyr Ala Ala Asp His Gly Ile Asp Pro Ala Phe Leu Arg Thr	90
CTG TAC GAC ACG ATC ATC ACC GAG ACC TGC CGC CTC GAG GAC GAG TGG ATC GCC	324
Leu Tyr Asp Thr Ile Ile Thr Glu Thr Cys Arg Leu Glu Asp Glu Trp Ile Ala	108
TCC GGC GGC GCC CCC GTC CCC ACG CCC GTG CAC GCG TCC GCG TCC GCG CGG GGG	378
Ser Gly Gly Ala Pro Val Pro Thr Pro Val His Ala Ser Ala Ser Ala Arg Gly	126
GCC GTG TCG	387
Ala Val Ser	129

## INFORMATION FOR SEQ ID NO: 4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4496 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: no

(vi) ORIGINAL SOURCE:

pristinaespiralis

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4

10	20	30	40	50	60
CTCGAGCAGG	TGCCCCACCT	CGGCGGCAGC	GTGCGCGGGC	AGCGCGAACA	CCGGCAGCGC
70	80	90	100	110	120
GCCCAGACGG	AACAGCGCGA	AGCACACCGC	GACGAACTCG	GGCGTGTTCT	GCAGCTGCAC
130	140	150	160	170	180
CAGCACCCGC	TCGCCGGCGC	CGATCCCGCG	CGCCGCGAAC	CCCGCCGCCA	GCCGGTCGCA
190	200	210	220	230	240
CCAGCGGTCC	AGGGCACGGT	AGGTGACACG	GGAGCACCCG	TCCGCGCCGA	CCAGCGCCTC
250	260	270	280	290	300
CCGCTCGCCG	TACTGCTCCG	CCCAGCGGCC	CAGCAGCATG	CCCAGCGGCT	CGCCCCGCCA
310	320	330	340	350	360
GTAGCCGGCC	GCCCGGTACT	TCGCGGCCAC	ATCCTCGGGC	CAGGGAACGC	ATCCGTCCAG
370	380	390	400	410	420

CATCGTGGT	CCTTTCGGC	TCGTCCTCG	CGTCGCGCC	AGTGTGCGCA	GCGCCGTTGA
430	440	450	460	470	480
CACGCCGCTG	ATGCGCCGCG	CCCGCGCGCC	GCCGCTCCGT	CAGGAGCCGA	TCAGGGCGGC
490	500	510	520	530	540
GTCAGCCGGG	CCGGACAGGA	TGCCGCCAC	GGGGCCCGGC	ACACCGGGCC	GCGGCGACAG
550	560	570	580	590	600
CGGGCCGGCG	ACCGGCAGGC	CGACACCACG	CACGGACGAG	AAGAAACAAC	ACAAGGGGAG
610	620	630	640	650	660
CACCCGATGG	AGACCTGGGT	CCTGGGCCGG	CGCGACGTCT	CCGAGGTGGT	GGCCGCCGTC
670	680	690	700	710	720
GGCCGCGACG	AACTCATGCG	CCGCATCATC	GACCGCCTCA	CCGGCGGACT	GGCCGAGATC
730	740	750	760	770	780
GGCCCGGGCG	AGCGGCACCT	GTCCCCGCTG	CGCGGCGGAC	TGGAACGCAG	CGAACCCGTC
790	800	810	820	830	840
CCCGGCATCT	GGGAATGGAT	GCCGCACCGC	GAACCCGGCG	ACCACATCAC	CCTCAAGACC
850	860	870	880	890	900
GTCGGCTACA	GCCCCGCCAA	CCCCGGCCGC	TTCGGCTGTC	CGACCATCCT	GGGCACCGTC
910	920	930	940	950	960
GCCCCGCTACG	ACGACACCAC	CGGCGCCCTG	ACCGCCCTGA	TGGACGGCGT	GCTGCTCACC
970	980	990	1000	1010	1020
GCCCTGCGCA	CCGGCGCCGC	CTCCGCCGTC	GCCTCCCGCC	TGCTGGCCCG	CCCCGACAGC
1030	1040	1050	1060	1070	1080
CACACCCTGG	GACTGATCGG	CACCGGCGCC	CAGGCCGTCA	CCCAACTGCA	CGCCCTGTCC
1090	1100	1110	1120	1130	1140
CTGGTACTGC	CCCTGCAACG	GGCCCTGGTG	TGGGACACCG	ACCCCGCCCA	CCGGGAAAGC
1150	1160	1170	1180	1190	1200
TTGCCCCGGC	GCGCCGCGTT	CACCGCGCTC	AGCGTCGAGA	TCGCCGAGCC	CGCCCCGATC
1210	1220	1230	1240	1250	1260
GCCGCCGAGG	CCGACGTCAT	CTCCACCGCC	ACCTCGGTAG	CCGTCGGCCA	GGGCCCGGTC
1270	1280	1290	1300	1310	1320
CTGCCCCACA	CCGGCGTCCG	CGAGCACCTG	CACATCAACG	CCGTCGGCGC	GGACCTCGTC
1330	1340	1350	1360	1370	1380
GGCAAGACGG	AACTGCCGCT	CGGCCTGCTC	GAGCGGGCGT	TCGTCAACGC	CGACCACCCC
1390	1400	1410	1420	1430	1440
GAGCAGGCGC	TGCGCGAGGG	CGAGTGCCAG	CAACTCTCCG	CCGACCGGCT	CGGCCCGCAG
1450	1460	1470	1480	1490	1500
CTGGCCCAAC	TGTGCGCCGA	CCCGGCGGCC	GCCGCCGGCC	GGCAGGACAC	CCTGAGCGTC
1510	1520	1530	1540	1550	1560
TTCGACTCCA	CCGGCTTCGC	CTTCGAGGAC	GCCCTGGCGA	TGGAAGTGTT	CCTCGAGGCC
1570	1580	1590	1600	1610	1620
GCCGCCGAAC	GGGACCTGGG	CATCCGGGTG	GGCATCGAAC	ACCACCCCGG	CGACGCCCTG
1630	1640	1650	1660	1670	1680
GACCCCTACG	CCCTCCAGCC	CCTGCCCTG	CCCCTGGCCG	CCCCCGCCCA	CTGACCCCCC
1690	1700	1710	1720	1730	1740
CCTTTTTTCG	GGACCCCCGC	TCTTTTTTCGA	GAACCCCGCC	CGGCCCGGCC	GGCCCTCCTC

1750	1760	1770	1780	1790	1800
CCGCCGGCCC	CCATGCCCGG	CCGGGCGGGG	GCACCCACGA	CGCCCTCGCG	AGGAGAGAGA
1810	1820	1830	1840	1850	1860
TGCCCCCCAC	CCCCCGGGCC	ACCACCGACG	ACGGCGGGCG	TGAACTGCTC	GCCTGGCTGC
1870	1880	1890	1900	1910	1920
GCGAGATGCG	CCACCACCAC	CCCGTCCACG	AGGACGAATA	CGGTGCCTTC	CACGTCTTCC
1930	1940	1950	1960	1970	1980
GGCACGCCGA	CGTCCTCACC	GTCGCCTCCG	ACCCCGGCGT	CTACTCCTCC	CAGCTCAGCC
1990	2000	2010	2020	2030	2040
GGCTACGGCC	CGGCTCCCLAG	GCGTTGAGCG	AACAGATCCT	GTCGGTCATC	GACCCGCCGA
2050	2060	2070	2080	2090	2100
TGCACCGCAC	CCTGCGCCGC	CTGGTCAGCC	AGGCCTTCAC	CCCCCGCACC	GTCGCCGACC
2110	2120	2130	2140	2150	2160
TCGAACCACG	CGTCACCGAA	CTGGCCGGGC	AACTGCTCGA	CGCCGTCGAC	GGCGACACGT
2170	2180	2190	2200	2210	2220
TCGACCTCGT	CGCCGACTTC	GCCTACCCGC	TGCCCGTGAT	CGTGATCGCC	GAACCTCCTG
2230	2240	2250	2260	2270	2280
GCGTGCCGCC	CGCCGACCGC	ACCCTGTTCC	GCTCCTGGTC	CGACCGGATG	CTGCAGATGC
2290	2300	2310	2320	2330	2340
AGGTGCGCGA	CCCCGGCGAC	ATGCAGTTCC	GCGACGACGC	CGACGAGGAC	TACCAACGCC
2350	2360	2370	2380	2390	2400
TCGTCAAAGA	ACCCATGCGC	GCCATGCACG	CCTACCTCCA	CGACCACGTC	ACCGACCGCC
2410	2420	2430	2440	2450	2460
GCGCCCGCCC	CGCGAACGAC	CTGATCTCCG	CACTCGTCGC	CGCCCGCGTG	GAGGGCGAAC
2470	2480	2490	2500	2510	2520
GACTCACCGA	CGAGCAGATC	GTCGAATTCC	GGGCGCTGCT	GCTGATGGCC	GGCCACGTCT
2530	2540	2550	2560	2570	2580
CCACCTCCAT	GCTGCTCGGC	AACACCGTGC	TGTGCCTGAA	GGACCACCCC	CGGGCCGAGG
2590	2600	2610	2620	2630	2640
CGGCCGCGCG	CGCCGACCGG	TCCCTGATCC	CCGCCCTGAT	CGAAGAAGTA	CTGCGGGCTGC
2650	2660	2670	2680	2690	2700
GGCCGCGGAT	CACCGTCATG	GCCCGCGTCA	CCACCAAGGA	CACCGTCCTC	GCCGGCACCA
2710	2720	2730	2740	2750	2760
CCATCCCCGC	CGGACGCATG	GTCGTGCCCT	CCCTGCTGTC	CGCCAACCAC	GACGAACAGG
2770	2780	2790	2800	2810	2820
TCTTCACCGA	CCCCGACCAC	CTCGACCTCG	CCCGCGAAGG	CCGCCAGATC	GCCTTCGGCC
2830	2840	2850	2860	2870	2880
ACGGCATCCA	CTACTGCCTG	GGCGCCCCGC	TGCGCCGCGT	GGAGGGCCGC	ATCGCCCTGG
2890	2900	2910	2920	2930	2940
AAGCCCTCTT	CGACCGATTG	CCCGACTTCT	CGCCACCGA	CGGCGCAAAA	CTGCGCTACC
2950	2960	2970	2980	2990	3000
ACCGCGACGG	ACTGTTCCGC	GTCAAGAACC	TGCCGCTGAC	CGTACGGCGC	GGCTGACACA
3010	3020	3030	3040	3050	3060
GACAAGGGGG	CCACCTGGTG	CGCACCGTGC	GAACCCTGCT	GATCGACAAC	TACGACTCGT

3070	3080	3090	3100	3110	3120
TCACCTACAA	CCTCTTCCAG	ATGCTGGCCG	AGGTGAACGG	CGCCGCTCCG	CTCGTCGTCC
3130	3140	3150	3160	3170	3180
GCAACGACGA	CACCCGCACC	TGGCAGGCCC	TGGCGCCGGG	CGACTTCGAC	AACGTCGTCG
3190	3200	3210	3220	3230	3240
TCTCACCCGG	CCCCGGCCAC	CCCGCCACCG	ACACCGACCT	GGGCCTCAGC	CGCCGGGTGA
3250	3260	3270	3280	3290	3300
TCACCGAATG	GGACCTGCCG	CTGCTCGGGG	TGTGCCTGGG	CCACCAGGCC	CTGTGCCTGC
3310	3320	3330	3340	3350	3360
TCGCCGGCGC	CGCCGTCGTC	CACGCACCCG	AACCCTTTCA	CGGCCGCACC	AGCGACATCC
3370	3380	3390	3400	3410	3420
GCCACGACGG	GCAGGGCCTG	TTCGCGAACA	TCCCTTCCCC	GCTGACCGTG	GTCCGCTACC
3430	3440	3450	3460	3470	3480
ACTCGCTGAC	CGTCCGGCAA	CTGCCCGCCG	ACCTGCGCGC	CACCGCCAC	ACCGCCGACG
3490	3500	3510	3520	3530	3540
GGCAGCTGAT	GGCCGTCGCC	CACCGCCACC	TGCCCGCCTT	CGGCGTGCAG	TTCACCCCG
3550	3560	3570	3580	3590	3600
AATCGATCAG	CAGCGAACAC	GGCCACCGGA	TGCTGCCAA	CTTCCGCGAC	CTGTCCCTGC
3610	3620	3630	3640	3650	3660
GCGCGGCCGG	CCACCGCCCC	CCGCACACCG	AACGCATACC	CGCACCCGCA	CCCCCCCCCG
3670	3680	3690	3700	3710	3720
CCCCCGCCCC	CGCACCGGCA	CCGCCCGCGT	CCGCGCCGGT	GGGGGAGTAC	CGGCTGCATG
3730	3740	3750	3760	3770	3780
TGCGCGAGGT	CGCCTGCGTG	CCCGACGCGG	ACGCCGCGTT	CACCGCCCTG	TTCGCCGACG
3790	3800	3810	3820	3830	3840
CCCCGGCCCC	GTTCTGGCTC	GACAGCAGCC	GCGTCGAGCC	GGGCCTCGCC	CGCTTCACCT
3850	3860	3870	3880	3890	3900
TCCTCGGCGC	CCCCGCCGGC	CCGCTCGGCG	AACAGATCAC	CTACGACGTC	GCCGACCGGG
3910	3920	3930	3940	3950	3960
CCGTGCGCGT	CAAGGACGGT	TCAGGCGGCG	AGACCCGCCG	GCCCGGCACC	CTCTTCGACC
3970	3980	3990	4000	4010	4020
ACCTGGAACA	CGAACTGGCC	GCCCGCGCCC	TGCCCGCCAC	CGGCCTGCCC	TTCGAGTTCA
4030	4040	4050	4060	4070	4080
ACCTCGGCTA	CGTCGGCTAC	CTCGGCTACG	AGACCAAGGC	CGACAGCGGC	GGCGAGGACG
4090	4100	4110	4120	4130	4140
CCCACCGCGG	CGAACTGCCC	GACGGCGCCT	TCATGTTGCG	CGACCGGATG	CTCGCCCTCG
4150	4160	4170	4180	4190	4200
ACCACGAACA	GGGGCGGGCC	TGGCTCCTGG	CACTGAGCAG	CACCCGACGG	CCCCCCACCG
4210	4220	4230	4240	4250	4260
CACCCGCGCG	CGAACGCTGG	CTCACCGACG	CCGCCCCGAC	CCTCGCCACC	ACCGCCCCCG
4270	4280	4290	4300	4310	4320
GCCCCGCCCTT	CACCCTGCTG	CCCGACGACC	AACTGCCCCG	CCTGGACGTC	CACTACCGCC
4330	4340	4350	4360	4370	4380
ACAGCCTGCC	CCGCTACCGG	GAAGTGGTCG	AGGAATGCCG	CCGCCTGATC	ACCGACGGCG
4390	4400	4410	4420	4430	4440
AGACCTACGA	GGTGTGCCTG	ACGAACATGC	TCCGGGTGCC	CGGCCGGATC	GACCCGCTCA
4450	4460	4470	4480	4490	
CCGCCTACCG	CGCCCTGCGC	ACCGTCAGCC	CCGCCCCCTA	CGCCCGCTAC	CTGCAG

```
(i)      SEQUENCE CHARACTERISTICS:
          (A)  LENGTH: 1065 base pairs
          (B)  TYPE: nucleic acid
          (C)  STRANDEDNESS: double
          (D)  TOPOLOGY: linear
```

(iii) HYPOTHETICAL: no

10 (vi) ORIGINAL SOURCE:  
(A) ORGANISM: *Streptomyces*  
*pristinaespiralis*

ATG	GAG	ACC	TGG	GTC	CTG	GGC	CGG	CGC	GAC	GTC	GCC	GAG	GTG	GTG	GCC	GCC	GTC	54
Met	Glu	Thr	Trp	Val	Leu	Gly	Arg	Arg	Asp	Val	Ala	Glu	Val	Val	Ala	Ala	Val	18
GGC	CGC	GAC	GAA	CTC	ATG	CGC	CGC	ATC	ATC	GAC	CGC	CTC	ACC	GGC	GGA	CTG	GCC	108
Gly	Arg	Asp	Glu	Leu	Met	Arg	Arg	Ile	Ile	Asp	Arg	Leu	Thr	Gly	Gly	Leu	Ala	36
GAG	ATC	GGC	CGC	GGC	GAG	CGG	CAC	CTG	TCC	CCG	CTG	CGC	GGC	GGA	CTG	GAA	CGC	162
Glu	Ile	Gly	Arg	Gly	Glu	Arg	His	Leu	Ser	Pro	Leu	Arg	Gly	Gly	Leu	Glu	Arg	54
AGC	GAA	CCC	GTG	CCC	GGC	ATC	TGG	GAA	TGG	ATG	CCG	CAC	CGC	GAA	CCC	GGC	GAC	216
Ser	Glu	Pro	Val	Pro	Gly	Ile	Trp	Glu	Trp	Met	Pro	His	Arg	Glu	Pro	Gly	Asp	72
CAC	ATC	ACC	CTC	AAG	ACC	GTC	GGC	TAC	AGC	CCC	GCC	AAC	CCC	GGC	CGC	TTC	GGC	270
His	Ile	Thr	Leu	Lys	Thr	Val	Gly	Tyr	Ser	Pro	Ala	Asn	Pro	Gly	Arg	Phe	Gly	90
CTG	CCG	ACC	ATC	CTG	GGC	ACC	GTC	GCC	CGC	TAC	GAC	GAC	ACC	ACC	GGC	GCC	CTG	324
Leu	Pro	Thr	Ile	Leu	Gly	Thr	Val	Ala	Arg	Tyr	Asp	Asp	Thr	Thr	Gly	Ala	Leu	108
ACC	GCC	CTG	ATG	GAC	GGC	GTG	CTG	CTC	ACC	GCC	CTG	CGC	ACC	GGC	GCC	GCC	TCC	378
Thr	Ala	Leu	Met	Asp	Gly	Val	Leu	Leu	Thr	Ala	Leu	Arg	Thr	Gly	Ala	Ala	Ser	126
GCC	GTC	GCC	TCC	CGC	CTG	CTG	GCC	CGC	CCC	GAC	AGC	CAC	ACC	CTG	GGA	CTG	ATC	432
Ala	Val	Ala	Ser	Arg	Leu	Leu	Ala	Arg	Pro	Asp	Ser	His	Thr	Leu	Gly	Leu	Ile	144
GGC	ACC	GGC	GCC	CAG	GCC	GTC	ACC	CAA	CTG	CAC	GCC	CTG	TCC	CTG	GTA	CTG	CCC	486
Gly	Thr	Gly	Ala	Gln	Ala	Val	Thr	Gln	Leu	His	Ala	Leu	Ser	Leu	Val	Leu	Pro	162

CTG CAA CGG GCC CTG GTG TGG GAC ACC GAC CCC GCC CAC CGG GAA AGC TTC GCC	540
Leu Gln Arg Ala Leu Val Trp Asp Thr Asp Pro Ala His Arg Glu Ser Phe Ala	180
CGG CGC GCC GCG TTC ACC GGC GTC AGC GTC GAG ATC GCC GAG CCC GCC CGG ATC	594
Arg Arg Ala Ala Phe Thr Gly Val Ser Val Glu Ile Ala Glu Pro Ala Arg Ile	198
GCC GCC GAG GCC GAC GTC ATC TCC ACC GCC ACC TCG GTA GCC GTC GGC CAG GGC	648
Ala Ala Glu Ala Asp Val Ile Ser Thr Ala Thr Ser Val Ala Val Gly Gln Gly	216
CCG GTC CTG CCC GAC ACC GGC GTC CGC GAG CAC CTG CAC ATC AAC GCC GTC GGC	702
Pro Val Leu Pro Asp Thr Gly Val Arg Glu His Leu His Ile Asn Ala Val Gly	234
GCG GAC CTC GTC GGC AAG ACG GAA CTG CCG CTC GGC CTG CTC GAG CGG GCG TTC	756
Ala Asp Leu Val Gly Lys Thr Glu Leu Pro Leu Gly Leu Leu Glu Arg Ala Phe	252
GTC ACC GCC GAC CAC CCC GAG CAG GCG CTG CGC GAG GGC GAG TGC CAG CAA CTC	810
Val Thr Ala Asp His Pro Glu Gln Ala Leu Arg Glu Gly Glu Cys Gln Gln Leu	270
TCC GCC GAC CGG CTC GGC CCG CAG CTG GCC CAC CTG TGC GCC GAC CCG GCG GCC	864
Ser Ala Asp Arg Leu Gly Pro Gln Leu Ala His Leu Cys Ala Asp Pro Ala Ala	288
GCC GCC GGC CGG CAG GAC ACC CTG AGC GTC TTC GAC TCC ACC GGC TTC GCC TTC	918
Ala Ala Gly Arg Gln Asp Thr Leu Ser Val Phe Asp Ser Thr Gly Phe Ala Phe	306
GAG GAC GCC CTG GCG ATG GAA GTG TTC CTC GAG GCC GCC GCC GAA CGG GAC CTG	972
Glu Asp Ala Leu Ala Met Glu Val Phe Leu Glu Ala Ala Ala Glu Arg Asp Leu	324
GGC ATC CGG GTG GGC ATC GAA CAC CAC CCC GGC GAC GCC CTG GAC CCC TAC GCC	1026
Gly Ile Arg Val Gly Ile Glu His His Pro Gly Asp Ala Leu Asp Pro Tyr Ala	342
CTC CAG CCC CTG CCC CTG CCC CTG GCC GCC CCC GCC CAC	1065
Leu Gln Pro Leu Pro Leu Pro Leu Ala Ala Pro Ala His	355

## (7) INFORMATION FOR SEQ ID NO: 6:

## (i) SEQUENCE CHARACTERISTICS:

- 5
- (A) LENGTH: 1194 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: no

## (iii) ANTISENSE: no

## 10 (vi) ORIGINAL SOURCE:

pristinaespiralis

TGG Met	CCC Pro	CCC Pro	ACC Thr	CCC Pro	CGG Arg	CCC Pro	ACC Thr	ACC Thr	GAC Asp	GAC Asp	GGC Gly	GGC Gly	CGT Arg	GAA Glu	CTG Leu	CTC Leu	GCC Ala	54 18
TGG Trp	CTG Leu	CGC Arg	GAG Glu	ATG Met	CGC Arg	CAC His	CAC His	CAC His	CCC Pro	GTC Val	CAC His	GAG Glu	GAC Asp	GAA Glu	TAC Tyr	GGT Gly	GCC Ala	108 36
TTC Phe	CAC His	GTC Val	TTC Phe	CGG Arg	CAC His	GCC Ala	GAC Asp	GTC Val	CTC Leu	ACC Thr	GTC Val	GCC Ala	TCC Ser	GAC Asp	CCC Pro	GGC Gly	GTC Val	162 54
TAC Tyr	TCC Ser	TCC Ser	CAG Gln	CTC Leu	AGC Ser	CGG Arg	CTA Leu	CGG Arg	CCC Pro	GGC Gly	TCC Ser	CAG Gln	GGC Ala	TTG Leu	AGC Ser	GAA Glu	CAG Gln	216 72
ATC Ile	CTG Leu	TCG Ser	GTC Val	ATC Ile	GAC Asp	CCG Pro	CCG Pro	ATG Met	CAC His	CGC Arg	ACC Thr	CTG Leu	CGC Arg	CGC Arg	CTG Leu	GTC Val	AGC Ser	270 90
CAG Gln	GCC Ala	TTC Phe	ACC Thr	CCC Pro	CGC Arg	ACC Thr	GTC Val	GCC Ala	GAC Asp	CTC Leu	GAA Glu	CCA Pro	CGC Arg	GTC Val	ACC Thr	GAA Glu	CTG Leu	324 108
GCC Ala	GGG Gly	CAA Gln	CTG Leu	CTC Leu	GAC Asp	GCC Ala	GTC Val	GAC Asp	GGC Gly	GAC Asp	ACG Thr	TTC Phe	GAC Asp	CTC Leu	GTC Val	GCC Ala	GAC Asp	378 126
TTC Phe	GCC Ala	TAC Tyr	CGG Pro	CTG Leu	CCC Pro	GTG Val	ATC Ile	GTG Val	ATC Ile	GCC Ala	GAA Glu	CTC Leu	CTC Leu	GGC Gly	GTG Val	CCG Pro	CCC Pro	432 144
GCC Ala	GAC Asp	CGC Arg	ACC Thr	CTG Leu	TTC Phe	CGC Arg	TCC Ser	TGG Trp	TCC Ser	GAC Asp	CGG Arg	ATG Met	CTG Leu	CAG Gln	ATG Met	CAG Gln	GTC Val	486 162
GCC Ala	GAC Asp	CCG Pro	GCG Ala	GAC Asp	ATG Met	CAG Gln	TTC Phe	GGC Gly	GAC Asp	GAC Asp	GCC Ala	GAC Asp	GAG Glu	GAC Asp	TAC Tyr	CAA Gln	CGC Arg	540 180
CTC Leu	GTC Val	AAA Lys	GAA Glu	CCC Pro	ATG Met	CGC Arg	GCC Ala	ATG Met	CAC His	GCC Ala	TAC Tyr	CTC Leu	CAC His	GAC Asp	CAC His	GTC Val	ACC Thr	594 198
GAC Asp	CGC Arg	CGC Arg	GCC Ala	CGC Arg	CCC Pro	GCG Ala	AAC Asn	GAC Asp	CTG Leu	ATC Ile	TCC Ser	GCA Ala	CTC Leu	GTC Val	GCC Ala	GCC Ala	CGC Arg	648 216
GTG Val	GAG Glu	GGC Gly	GAA Glu	CGA Arg	CTC Leu	ACC Thr	GAC Asp	GAG Glu	CAG Gln	ATC Ile	GTC Val	GAA Glu	TTC Phe	GGG Gly	GCG Ala	CTG Leu	CTG Leu	702 234
CTG Leu	ATG Met	GCC Ala	GGC Gly	CAC His	GTC Val	TCC Ser	ACC Thr	TCC Ser	ATG Met	CTG Leu	CTC Leu	GGC Gly	AAC Asn	ACC Thr	GTG Val	CTG Leu	TGC Cys	756 252
CTG Leu	AAG Lys	GAC Asp	CAC His	CCC Pro	CGG Arg	GCC Ala	GAG Glu	GCC Ala	GCC Ala	GCC Ala	CGC Arg	GCC Ala	GAC Asp	CGG Arg	TCC Ser	CTG Leu	ATC Ile	810 270
CCC Pro	GCC Ala	CTG Leu	ATC Ile	GAA Glu	GAA Glu	GTA Val	CTG Leu	CGG Arg	CTC Leu	CGG Arg	CCG Pro	CCG Pro	ATC Ile	ACC Thr	GTC Val	ATG Met	GCC Ala	864 288



CGC GTC ACC ACC AAG GAC ACC GTC CTC GCC GGC ACC ACC ATC CCC GCC GGA CGC 918  
 Arg Val Thr Thr Lys Asp Thr Val Leu Ala Gly Thr Thr Ile Pro Ala Gly Arg 306

ATG GTC CTC CCC TCC CTG CTG TCC GCC AAC CAC GAC GAA CAG GTC TTC ACC GAC 972  
 Met Val Val Pro Ser Leu Leu Ser Ala Asn His Asp Glu Gln Val Phe Thr Asp 324

CCC GAC CAC CTC GAC CTC GCC CGC GAA GGC CGC CAG ATC GCC TTC GGC CAC GGC 1026  
 Pro Asp His Leu Asp Leu Ala Arg Glu Gly Arg Gln Ile Ala Phe Gly His Gly 342

ATC CAC TAC TGC CTG GGC GCC CCG CTC GCC CGC CTG GAG GGC CGC ATC GCC CTG 1080  
 Ile His Tyr Cys Leu Gly Ala Pro Leu Ala Arg Leu Glu Gly Arg Ile Ala Leu 360

GAA GCC CTC TTC GAC CGA TTC CCC GAC TTC TCG CCC ACC GAC GGC GCA AAA CTG 1134  
 Glu Ala Leu Phe Asp Arg Phe Pro Asp Phe Ser Pro Thr Asp Gly Ala Lys Leu 378

CGC TAC CAC CGC GAC GGA CTG TTC GGC GTC AAG AAC CTG CCG CTG ACC GTA CGG 1188  
 Arg Tyr His Arg Asp Gly Leu Phe Gly Val Lys Asn Leu Pro Leu Thr Val Arg 396

CGC GGC 1194  
 Arg Gly 398

## (8) INFORMATION FOR SEQ ID NO: 7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1561 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: no

## (iii) ANTISENSE: no

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: *Streptomyces pristinaespiralis*

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7

10 20 30 40 50 60  
 AAGCTTCCCG ACCGGGTGGA GGTCTGTCGAC GCGTTCCCGC TGACCGGCCT CAACAAGGTC

70 80 90 100 110 120  
 GACAAGAAGG CCCTGGCGGC CGACATCGCC GCCAAGACCG CCCCCACCCG CCCCACCACC

130 140 150 160 170 180  
 GCCGGCCACG GCCCGACCAC GGACGGCGAT ACGGCCGGTG GGGGTGGGTC CGCGGGCGGG

190 200 210 220 230 240  
 GTGACGGCCG CCGGTGGCGG GCGGGAGGAG GCGGCGTGAG CGGGCCCCGG CCGAGGGCGG

250 260 270 280 290 300  
 GCTACCGGGT GCCGTTCGCG CGACGCGGTT CGGTGGTGGG CGAGGCGGAC CTGGCGGCGC

3

5

(A) LENGTH: 1233 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: no

10

(A) ORGANISM: Streptomyces  
pristinaespiralis

GTG	CCG	TTC	GCG	CGA	CGC	GGT	TCG	GTG	GTG	GGC	GAG	GCG	GAC	CTG	GCG	GCG	CTG	54
Val	Pro	Phe	Ala	Arg	Arg	Gly	Ser	Val	Val	Gly	Glu	Ala	Asp	Leu	Ala	Ala	Leu	18
GGC	GAA	CTG	GTC	CGC	TCG	GGC	CGG	TCG	CTG	ACG	TCG	GGG	GTG	TGG	CGG	GAG	CGG	108
Gly	Glu	Leu	Val	Arg	Ser	Gly	Arg	Ser	Leu	Thr	Ser	Gly	Val	Trp	Arg	Glu	Arg	36
TTC	GAG	GAA	CAG	TTC	GCC	CGC	CTG	ACC	GGC	GCC	CGG	CAC	GCG	CTC	AGT	GTC	ACC	162
Phe	Glu	Glu	Gln	Phe	Ala	Arg	Leu	Thr	Gly	Ala	Arg	His	Ala	Leu	Ser	Val	Thr	54
AGC	GGC	ACC	GTC	GCG	CTG	GAA	CTG	GCG	GTG	CGG	ATG	CTG	GAC	CTG	GCG	CCG	GGC	216
Ser	Gly	Thr	Val	Ala	Leu	Glu	Leu	Ala	Val	Arg	Met	Leu	Asp	Leu	Ala	Pro	Gly	72
GAC	GAG	GTG	ATC	GCC	ACC	CCG	CAG	ACG	TTC	CAG	GCG	ACG	GTG	CAG	CCG	CTG	CTC	270
Asp	Glu	Val	Ile	Ala	Thr	Pro	Gln	Thr	Phe	Gln	Ala	Thr	Val	Gln	Pro	Leu	Leu	90
GAC	CAC	GAC	GTG	CGG	CTG	CGG	TTC	TGC	GAC	ATC	GAC	CCG	GAC	ACC	CTC	AAC	CTC	324
Asp	His	Asp	Val	Arg	Leu	Arg	Phe	Cys	Asp	Ile	Asp	Pro	Asp	Thr	Leu	Asn	Leu	108
GAC	CCG	GCG	GTG	CTG	GAG	ACG	CTG	ATC	ACC	GAC	CGC	ACC	CGG	GCG	ATC	CTG	CTC	378
Asp	Pro	Ala	Val	Leu	Glu	Thr	Leu	Ile	Thr	Asp	Arg	Thr	Arg	Ala	Ile	Leu	Leu	126
GTC	CAC	TAC	GGC	GGC	AAC	CCG	GCC	GAC	ATG	GAC	CGC	ATC	ATG	GCC	CTG	GCC	CGC	432
Val	His	Tyr	Gly	Gly	Asn	Pro	Ala	Asp	Met	Asp	Arg	Ile	Met	Ala	Leu	Ala	Arg	144
AAG	CGC	GGC	ATC	ATC	GTC	GTC	GAG	GAC	AGC	GCG	CAC	GCG	CTG	GGC	GCC	GTG	TAC	486
Lys	Arg	Gly	Ile	Ile	Val	Val	Glu	Asp	Ser	Ala	His	Ala	Leu	Gly	Ala	Val	Tyr	162
CGG	GGG	CGG	CGG	CCG	GGG	GCA	CTG	GCG	GAC	ATC	GGC	TGC	TTC	ACT	TTC	CAC	TCC	540
Arg	Gly	Arg	Arg	Pro	Gly	Ala	Leu	Ala	Asp	Ile	Gly	Cys	Phe	Thr	Phe	His	Ser	180

ACG	AAG	AAC	ATC	ACC	ACC	CTC	GGC	GAG	GGC	GGC	ATG	ATC	ACC	CTG	TCG	CGT	GAC	594
Thr	Lys	Asn	Ile	Thr	Thr	Leu	Gly	Glu	Gly	Gly	Met	Ile	Thr	Leu	Ser	Arg	Asp	198
GAG	TGG	GCC	CAG	CGG	GTG	GGA	CGT	ATC	CGC	GAC	AAC	GAG	GCC	GAC	GGC	GTG	TAC	648
Glu	Trp	Ala	Gln	Arg	Val	Gly	Arg	Ile	Arg	Asp	Asn	Glu	Ala	Asp	Gly	Val	Tyr	216
GCG	GCG	CTG	CCG	GAC	TCC	GCG	CGG	GCG	GGT	GCT	CCG	GCG	CTG	CTG	CCG	TGG	ATG	702
Ala	Ala	Leu	Pro	Asp	Ser	Ala	Arg	Ala	Gly	Ala	Pro	Ala	Leu	Leu	Pro	Trp	Met	234
AAG	TTC	GCG	GAG	GGT	GTG	TAC	GGT	CAC	CGG	GCG	GTC	GGG	GTC	CGC	GGG	GCG	GGC	756
Lys	Phe	Ala	Glu	Gly	Val	Tyr	Gly	His	Arg	Ala	Val	Gly	Val	Arg	Gly	Ala	Gly	252
ACG	AAC	GCG	ACG	ATG	TCG	GAG	GCG	GCG	GCG	GCG	GTG	GGC	GTG	GTG	CAA	CTG	GCG	810
Thr	Asn	Ala	Thr	Met	Ser	Glu	Ala	Ala	Ala	Ala	Val	Gly	Val	Val	Gln	Leu	Ala	270
TCG	CTG	GAG	CGG	TTC	GTG	GCC	CGG	CGC	CGG	AGC	ATC	GCG	CAG	CGG	CTG	GAC	GAG	864
Ser	Leu	Glu	Arg	Phe	Val	Ala	Arg	Arg	Arg	Ser	Ile	Ala	Gln	Arg	Leu	Asp	Glu	288
GCC	GTG	GCC	TCG	GTG	GCC	GGC	ACC	CGG	CTG	CAC	CGG	GCG	GCG	GCG	GAC	AGT	CTG	918
Ala	Val	Ala	Ser	Val	Ala	Gly	Thr	Arg	Leu	His	Arg	Ala	Ala	Ala	Asp	Ser	Leu	306
CAC	GCC	TAC	CAC	CTG	TAC	ACG	TTC	TTC	CTC	ACC	GGC	GGC	CGG	CAG	GTG	CGG	GAG	972
His	Ala	Tyr	His	Leu	Tyr	Thr	Phe	Phe	Leu	Thr	Gly	Gly	Arg	Gln	Val	Arg	Glu	324
CGG	TTC	GTG	CGC	GCC	CTG	GAC	CGG	CTG	GGT	GTG	GAG	GTC	CAG	TTG	CGG	TAC	TTC	1026
Arg	Phe	Val	Arg	Ala	Leu	Asp	Arg	Leu	Gly	Val	Glu	Val	Gln	Leu	Arg	Tyr	Phe	342
CCG	CTC	CAT	CTG	TCG	CCC	GAG	TGG	CGG	CTG	CGC	GGC	CAC	GGG	CCG	GGC	GAG	TGT	1080
Pro	Leu	His	Leu	Ser	Pro	Glu	Trp	Arg	Leu	Arg	Gly	His	Gly	Pro	Gly	Glu	Cys	360
CCG	ACG	GCC	GAA	CGG	GTC	TGG	TTC	GAG	GAG	CAC	ATG	AAC	CTG	CCG	TGC	CAT	CCC	1134
Pro	Thr	Ala	Glu	Arg	Val	Trp	Phe	Glu	Glu	His	Met	Asn	Leu	Pro	Cys	His	Pro	378
GGT	CTG	AGT	GAC	GGC	CAG	GTC	GAC	TAC	ATG	GTC	GAG	GCG	GTC	ACC	CGC	GCC	CTG	1188
Gly	Leu	Ser	Asp	Gly	Gln	Val	Asp	Tyr	Met	Val	Glu	Ala	Val	Thr	Arg	Ala	Leu	396
CAC	GAG	GCC	CAC	GGC	ACG	GGG	ACG	CGG	GTG	GCG	GCC	GGG	CAC	CTG				1233
His	Glu	Ala	His	Gly	Thr	Gly	Thr	Arg	Val	Ala	Ala	Gly	His	Leu				411